L Number	Hits	Search Text	DB	Time stamp
1	39337	fetus or fetal	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 09:49
6	17559	(fetus or fetal) and (insert$4 or implant$4)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 09:56
7	16561	((fetus or fetal) and (insert$4 or implant$4)) and human$4	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 09:56
8	2515	(((fetus or fetal) and (insert$4 or implant$4)) and human$4) and @py<=1994	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 10:29
9	3343	abort$4 with (method or process)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 10:00
10	150	(abort$4 with (method or process)) and (fetus or fetal)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 10:00
11	59	((abort$4 with (method or process)) and (fetus or fetal)) and (cut$4 or incis$4)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 10:02
12	332	((((fetus or fetal) and (insert$4 or implant$4)) and human$4) and @py<=1994) and (abort or abortion)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 10:31
13	18	((fetus or fetal) with abort$4) same (implant$4 or insert$4)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 10:35
14	2	("5360610").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 11:12
15	18	curette and abortion	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 10:56
16	0	(128/304).CCLS.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 11:12
26	3	("4883666" | "4962091" | "4994281").PN.	USPAT	2003/02/13 11:31
27	0	("implant with (human near (fetal or fetus))").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 11:40
29	157	(human near2 (fetal or fetus)) with (insert$4 or transplant$4)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 12:14
30	15	((human near2 (fetal or fetus)) with (insert$4 or transplant$4)) and @py<=1994	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 11:44
31	0	(human near2 (fetal or fetus)) with (graftt$4)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 11:50

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32	33	(human near2 (fetal or fetus)) with (graft$4)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 11:55
33	2	("5175103").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 11:55
34	121	(human near2 (embryo$5)) with (graft$4 or implant$4 or insert$4 or transplant$4)	USPAT; EPO; JPO; DERWENT; IBM_TDB	2003/02/13 12:16

Daniel Davis

25/9/41 (Item 41 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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00732672 BIOSIS NO.: 000052092740
IATROGENIC PARACERVICAL IMPLANTATION OF FETAL TISSUE DURING THERAPEUTIC ABORTION A CASE REPORT
AUTHOR: AYERS L R; DROSMAN S; SALTZSTEIN S L
JOURNAL: OBSTET GYNECOL 37 (5). 1971 755-760. 1971
FULL JOURNAL NAME: Obstetrics and Gynecology
CODEN: OBGNA
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11314 Chordate Body Regions-Abdomen (1970-)
12512 Pathology, General and Miscellaneous-Therapy (1971-)
16501 Reproductive System-General; Methods
BIOSYSTEMATIC CODES:
86215 Hominidae
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA):
Animals
Chordates
Vertebrates
Mammals
Primates
Humans

25/9/42 (Item 42 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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Utility of fragmented human fetal tissue as a potential dopaminergic brain graft in Parkinson's disease.
AUTHOR: Hogenesch R I(a); Staal M J; Kema I P; Buys C H C M; Go K G
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JOURNAL: Stereotactic and Functional Neurosurgery 61 (1):p1-11
ISSN: 1011-6125
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: There is increasing interest in the use of human **fetal** dopaminergic **tissue** as a source of striatal **transplant** in parkinsonian patients. This tissue is acquired by elective abortions. The possibilities of the use of this tissue were studied by macroscopical examination, cell-culturing followed by immunohistochemical staining and by high performance liquid chromatography. It turned out that 50% of the curettages obtained by **suction abortion** were too fragmented to reliably recognize the dopamine-containing area (ventral mesencephalon). Furthermore, **dissection** of the brainstem immediately after the **abortion** procedure seemed to be of utmost importance.

DESCRIPTORS:
MAJOR CONCEPTS: Development; Endocrine System (Chemical Coordination and Homeostasis); Metabolism; Nervous System (Neural Coordination); Neurology (Human Medicine, Medical Sciences); Pathology; Physiology; Surgery (Medical Sciences)
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia

Iatrogenic Paracervical Implantation of Fetal Tissue During Therapeutic Abortion.

A Case Report

LOREN R. AYERS, MD, STEVEN DROSMAN, MD and SIDNEY L.SALTZSTEIN, MD

Fetal parts were implanted in the paracervical tissue during a therapeutic abortion performed with a vacuum curette. Three months later, these parts presented as a 6-cm paracervical mass. Laparotomy and hysterectomy were necessary to elucidate fully the nature of the mass.

A PARACERVICAL MASS, thought to represent a tumor, proved to be fetal tissue implanted during a therapeutic abortion three months earlier. This complication of vacuum curettage has not been reported previously in the English-language literature.

CASE REPORT

CR, a 32-year-old female, was admitted to our Coronary Care Unit on July 3, 1969. Pulmonary embolism was confirmed by pulmonary arteriography and lung scan. She was pregnant for the sixth time; her LMP had been May 10, 1969. After her condition stabilized, a therapeutic abortion was recommended by her physician and approved by the Therapeutic Abortion Committee. The abortion was performed by transcervical suction on July 18, 1969. The uterus was approximately 10–12 weeks' gestational size, and a uterine sound entered to a depth of five inches. No adnexal or cervical masses were detected during a pelvic examination carried out while the patient was anesthetized. The suction curettage was performed without difficulty. Despite the fact that the patient was receiving heparin, postoperative vaginal bleeding was minimal. She was discharged on July 29, to continue Coumadin* therapy. She was to be readmitted for a hysterectomy.

On Aug 25, 1969, the patient had a normal menstrual period, lasting approximately five days. On Sept 20, she began very heavy vaginal bleeding with large clots and required at least 20 pads during the next 18 hours. On Sept 21 an examination by her physician disclosed a 6-cm mass in the region of the right adnexa; the uterus was felt to be enlarged very minimally. An injection of progesterone seemed to decrease the amount of vaginal bleeding, but the patient continued to spot until her readmission on Oct 11, 1969. At this time, Coumadin was discontinued and intravenous therapy with heparin was started. On Oct 18, the patient underwent a total abdominal hysterectomy and a 1.5-cm right paraovarian cyst was excised. During hysterectomy, a 3-cm mass was noted to be attached to the outside of the cervix. Both ovaries and fallopian tubes were normal except for the thin-walled paraovarian cyst. Because her postoperative course was complicated by a vaginal cuff hematoma and subcutaneous bleeding, heparin therapy was discontinued.

She was discharged approximately three weeks' postoperatively in satisfactory condition. When last seen (Feb 2, 1970), she still had pulmonary difficulties but no further gynecologic problems.

Pathologic Examination

The tissue received from the therapeutic abortion consisted of 70 g of spongy, red-tan tissue admixed with membranes and fetal parts. When representative sections were examined

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* Endo Laboratories Inc, 1000 Stewart Ave, Garden City, NY 11530.

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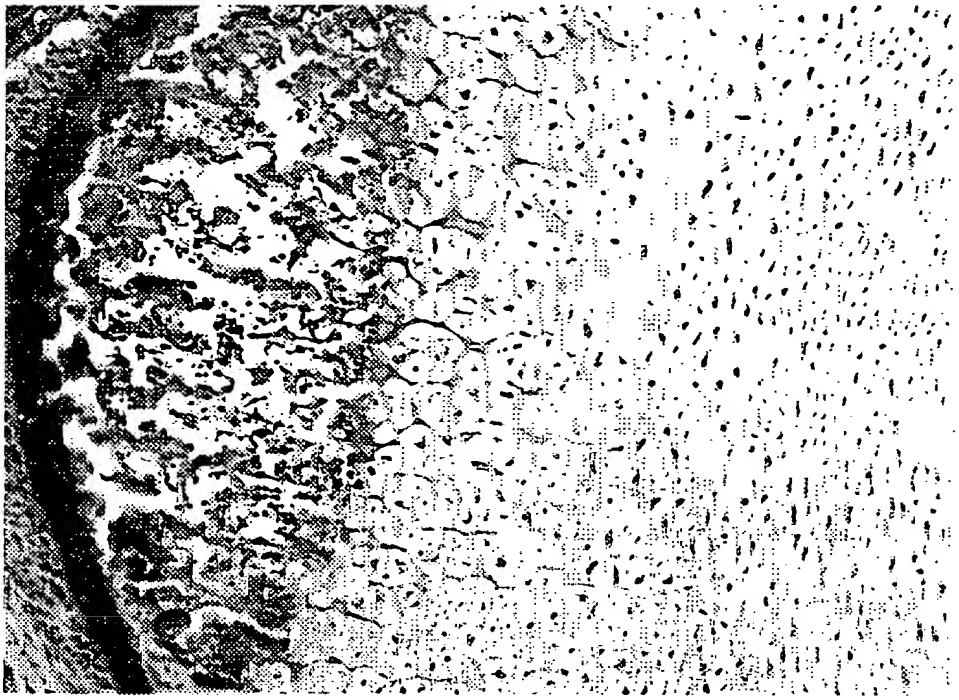

Fig 1. Fetal enchondral bone formation in tissue removed at vacuum curettage (H & E, enlarged from 25 ×).

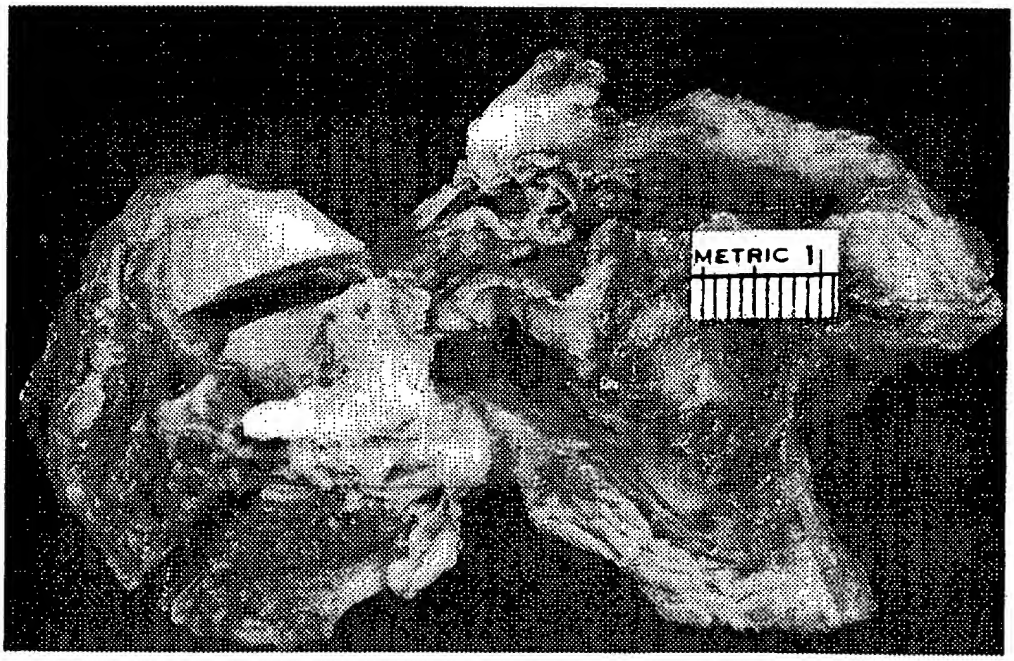

Fig 2. Paracervical mass removed during hysterectomy. The mass has been bivalved and laid open.

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microscopically, decidua, placental villi and various fetal tissues, including bone, were seen (Fig 1).

Three months later, a 11 × 5 × 3 cm uterus was received. The uterus appeared to be normal except for a 3-cm spherical, firm mass protruding laterally from the external surface of the cervix at a level corresponding to the internal os. The mass extended through all layers of the cervix and 0.5 cm into the endocervical canal. On sectioning, central necrosis and hemorrhage with some calcification were seen. (Fig 2). The presence of well-formed fetal structures in the mass was confirmed roentgenographically. (Fig 3). Microscopically, the mass was composed of necrotic decidua as well as fetal muscle, bone and cartilage. Enchondral bone formation in this bone tissue was at the same stage of development as that seen in tissue from the therapeutic abortion; intermembraneous bone formation possibly represented the fetal skull (Fig 4 and 5). No placental tissue or fetal membranes were seen in multiple sections. A chronic inflammatory reaction was also present. The fetal tissue and reaction approached the cervical epithelium in one portion, representing the 0.5 cm projection into the endocervical canal. However, the cervical epithelium here, as well as elsewhere in the cervix, was normal. Neither the glands nor the stroma were characterized by any of the changes associated with pregnancy. The remainder of the uterus was normal; a secretory endometrium was noted. The paraovarian cyst had a simple cuboidal epithelial lining.

DISCUSSION

Before this is accepted as an instance of iatrogenic implantation of fetal parts into the paracervical tissue, all other possibilities must be excluded. The presence of two normal ovaries, the location of the mass, and the degree of organization seen both microscopically and roentgenographically eliminate the possibility of a teratoid tumor. The absence of the mass at the time of vacuum curettage also militates against this.

That the paracervical mass represents an ectopic (cervical) pregnancy occurring after the therapeutic abortion is a second possibility. Cervical pregnancy has been reported as a sequel of induced abortion.[1] However, for the presumed paracervical pregnancy to have attained the observed degree of devel-

Fig 3. Roentgenogram of the paracervical mass. Fetal ribs, spine, skull, etc, are readily identifiable (enlarged 1.5 ×).

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Fig 4 (top). Fetal enchondral bone formation in tissue from paracervical mass. Resemblance to Fig 1 is apparent (H & E, enlarged from 25 ×). Fig 5 (bottom). Fetal membranous bone formation (? skull) in tissue from paracervical mass (H & E, enlarged from 25 ×).

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opment—ie, the same as that removed during the therapeutic abortion, the woman would have had to conceive almost immediately after the therapeutic abortion in July. Thus, the apparently normal menstrual period in August would have to be considered as bleeding during pregnancy. Moreover, cervical pregnancy implies a mucosal, rather than a paracervical, implantation site.[2-6] The mass in our patient was almost entirely external to the cervix with no involvement of the cervical mucosa. No changes of pregnancy were noted in the cervical glands, the cervical stroma or endometrium. The broken-up appearance of the fetal parts on the roentgenogram suggest strongly that the fetal parts were introduced forcibly into the paracervical tissue, rather than growing into that location, in a more or less normal manner, from the cervical canal. Finally, nothing resembling either a placenta or an amniotic cavity could be identified.

Another possibility is that the fetus in the paracervical tissue represented a twin to the one removed by the vacuum curette. Although this could explain the same degree of development in the intrauterine and paracervical tissue, all of the objections in the preceding paragraph may be raised against this possibility. Moreover, the paracervical mass was not palpated at the time of the therapeutic abortion.

The contention that the fetal tissues were implanted at the time of vacuum curettage is supported by evidence of a positive nature. We have seen several instances in which not all fetal tissues were removed at the time of curettage, and either passed spontaneously days later or required subsequent curettage. This has been reported by other authors.[7-12] The chance that the cervix might have been more subject to penetration than usual because of the heparin therapy, and, thus, allowed the introduction of fetal tissues by

Fig 6. Faxitron roentgenogram of vacuum curettements from another patient. The fetal head, trunk, pelvis, three extremities and other parts can be identified (enlarged 1.5 ×).

Fig 1 is
skull) in

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the curette must also be considered. The broken-up appearance of the fetal skeleton would fit best with the concept of forcible introduction.

Combining all of these observations, one can think of no logical way that the fetal parts could have been introduced into the paracervical tissue, except instrumentally, at the time of the vacuum curettage.

Prevention of this occurrence, and of the disconcerting but not necessarily dangerous retention of fetal tissues for some time after therapeutic abortion, requires a means of determining if all fetal tissues were indeed removed at the original procedure. We have tried to obtain roentgenograms of the removed tissue by utilizing a Faxitron® laboratory X-ray unit and Polaroid® film. With this technic, we can attempt to account for the majority of the fetus with very little additional work, and the results (Polaroid roentgenograms) can be made available to the obstetrician while the patient is still in the operating room, if desired (Fig 6). Copies of these roentgenograms can be included in the surgical pathology report. Unfortunately, the fetal skeleton is not sufficiently calcified before the twelfth week of gestation to give any degree of reliability by this method. After 12 weeks, technics of abortion other than vacuum curettage are used, and the problem of identifying the presence of the entire fetus becomes simple.

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Sep 15 1983

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Use of ultrasound in the prenatal diagnosis of congenital disorders
1982

Clinical Observations

Vacuum extraction: use in a small rural hospital

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GEORDIE B. FALLIS, MD, CCFP

The effectiveness of vacuum extraction with the Silastic Obstetrical Vacuum Cup (SOVC), which has a soft, malleable cup, was assessed by two family physicians in a small rural hospital. Vacuum extraction was attempted in 35 of 231 deliveries over an 18-month period, with an overall success rate of 66%. The main indications for vacuum extraction were fetal distress, followed by a prolonged second stage of labour and malrotation of the occiput. The efficiency of the technique improved with experience. The effects of vacuum extraction on the fetus and mother compared favourably with those reported in the literature. After introduction of the SOVC, the rate of primary cesarean section for cephalopelvic disproportion declined, as did the rate of forceps delivery. Despite careful antenatal screening and referral, and the availability of alternatives, delivery by vacuum extraction with the SOVC was found to be a useful and effective adjunct to obstetric practice.

L'efficacité de l'extraction sous vide à l'aide de la ventouse obstétricale Silastic (VOS), un instrument possédant une cupule molle et malléable, a été appréciée par deux médecins de famille dans un petit hôpital rural. Au cours d'une période de 18 mois l'extraction sous vide a été tentée dans 35 accouchements sur un total de 231, avec un taux de succès global de 66%. Les principales indications de l'extraction par ventouse furent la souffrance foetale, suivie de la prolongation du deuxième stade du travail et de la malrotation de l'occiput. L'efficacité de la technique s'améliorait avec l'expérience. Les effets de l'extraction par ventouse sur le foetus et sur la mère se sont comparés favorablement à ceux qui sont décrits dans la littérature. Avec l'utilisation de la VOS la fréquence des césariennes primaires dans les cas de disproportion céphalo-pelvienne s'est abaissée, de même que le nombre d'accouchements par application des forceps. En dépit d'un dépistage prénatal soigneux, de l'orientation de certains cas vers un établissement spécialisé et de la disponibilité d'autres moyens, l'accouchement par extraction sous vide à l'aide de la ventouse VOS s'est avérée un complément utile et efficace à la pratique de l'obstétrique.

Physicians in small rural hospitals must be prepared to deal expeditiously and safely with unexpected complications of labour and delivery.[1] However, restrictions in training and practice often make it difficult for them to maintain the necessary competence in operative techniques, particularly forceps deliveries.[2,3] Therefore, vacuum extraction, when used to correct malrotation, promote descent or do both seems most appropriate in remote hospitals.[4-7] The safety of vacuum extraction, originally proposed by Simpson[8] in 1849, has been enhanced by the recent introduction of devices with a soft, malleable cup, such as the Silastic Obstetrical Vacuum Cup (SOVC) (Dow Corning Corporation, Midland, Michigan).[9-11] We assessed the use of vacuum extraction with the SOVC at the Baie Verte Peninsula Health Centre (BVPHC), the only source of primary care for 12 000 Newfoundlanders living in 21 remote coastal villages. The 40-bed hospital, staffed by six to eight family physicians, is equipped to do cesarean sections, but, whenever possible, high-risk patients are referred to the nearest centre with specialized facilities, which is 180 km from the BVPHC.

Methods

The SOVC is trumpet-shaped, 208 mm long and made of a soft, translucent silicone elastomer. The diameter of the cup-shaped end is 65 mm (Fig. 1). Either a wall or a mechanical vacuum can be used, with a pressure of −200 to −650 mm Hg.

The study period began July 1, 1980, when the SOVC was first used at BVPHC, and ended Dec. 31, 1981. During this time 231 infants were delivered at the hospital, and 35 women at moderate to high risk, according to the provincial guidelines,[12] were transferred for delivery at a regional centre.

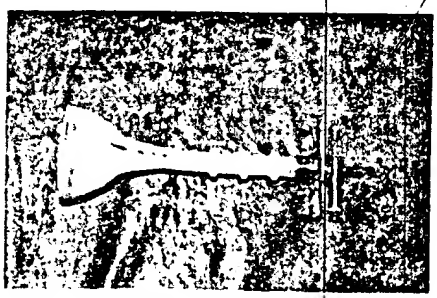

FIG. 1—Silastic Obstetrical Vacuum Cup.

From the Baie Verte Peninsula Health Centre, Baie Verte, Nfld.

Reprint requests to: Dr. Geordie B. Fallis, Baie Verte Peninsula Health Centre, Baie Verte, Nfld. A0K 1B0

The SOVC was used primarily by one of us (G.B.F.), usually as an alternative to Simpson's forceps, in expediting delivery. The indication for extraction was determined retrospectively from the physician's delivery record. The SOVC was applied to the vertex after rupture of the membranes; full cervical dilatation was not always a prerequisite. The application of the cup was considered to be high, mid, low-mid or low, according to Dennen's definition.[1] A wall vacuum with a pressure of −450 mm Hg was used, and traction was applied simultaneously with the uterine contractions. The outcome was recorded as "complete" (successful extraction), "incomplete" (correction of malrotation, or promotion of descent or both, but unsuccessful extraction) or "failed" (no effect). Another method of delivery was used if complete extraction was not accomplished within 20 minutes or if the cup had become detached from the vertex three times.

Results

The SOVC was used for 35 (15%) of the 231 deliveries (Table I). The indications for vacuum extraction are given in Table II. Approximately one third of the extractions were performed for fetal distress. Fetal distress was usually indicated if the amniotic fluid contained meconium or if there was a deceleration in the fetal heart rate, or both (sampling of the pH of scalp blood and electronic monitoring of the fetal heart rate were not available). Of the 11 extractions indicated by fetal distress 10 were complete; in 1 an incomplete extraction resulted in spontaneous delivery.

The outcome of the 35 applications of the SOVC is shown in Table III. Of the 12 deliveries in which vacuum extraction was unsuccessful, 5 were accomplished by cesarean section and 3 by forceps delivery, and 4 were spontaneous. Only the primigravidas required operative delivery.

The rate of complete extraction was decreased in the presence of malrotation, failure of descent or both, and was 50% if the presentation was other than occiput anterior, 50% when the SOVC was applied before full cervical dilatation and 33% when the SOVC was applied high or mid.

During the study period other staff physicians gradually became competent in using the SOVC. Of all the factors related to outcome, the experience of the operator was most consistently associated with complete extraction. The rate of incomplete or failed extraction was 53% (with spontaneous delivery occurring subsequently in four of nine cases) when the SOVC was applied by an inexperienced physician, compared with 17% (with delivery by cesarean section in all cases) when applied by an experienced physician.

Although epidural anesthesia was available, vacuum extraction was well tolerated with either a pudendal block or local infiltration. However, episiotomy and laceration involving the external anal sphincter was more likely with vacuum extraction than with spontaneous vaginal delivery. Postpartum hemorrhage occurred in 7% of the women who underwent vacuum extraction. The incidence of disorders characterized by fever was

Table III—Outcome of attempted vacuum extraction

	No. (and %) of deliveries		
	Parity		
Outcome	0	≥ 1	Total
Complete	14 (40)	9 (26)	23 (66)
Incomplete	3 (8)	1 (3)	4 (11)
Failed	8 (23)	0 (0)	8 (23)
Total	25 (71)	10 (29)	35 (100)

Table I—Method of delivery according to parity

	No. (and %) of deliveries		
	Parity		
Method of delivery	0	≥ 1	Total
Spontaneous vaginal	53 (23)	97 (42)	150 (65)
Cesarean section			
Primary	22 (10)	5 (2)	27 (12)
Repeat	0 (0)	21 (9)	21 (9)
Vacuum extraction with SOVC*	14 (6)	9 (4)	23 (10)
Forceps	7 (3)	2 (< 1)	9 (4)
Assisted breech	1 (< 1)	0 (0)	1 (< 1)
Total	97 (42)	134 (58)	231 (100)

*SOVC = Silastic Obstetrical Vacuum Cup.

Table II—Indications for vacuum extraction

	No. (and %) of deliveries			
	Parity			
Indication	0	≥ 1	Total	No. (and %) of complete extractions
Fetal distress	5 (14)	6 (17)	11 (32)	10 (91)
During first stage of labour	1 (3)	1 (3)	2 (6)	2 (100)
During second stage of labour	4 (11)	5 (14)	9 (26)	8 (89)
Prolonged second stage of labour	5 (14)	0 (0)	5 (14)	1 (20)
Deep transverse arrest	3 (8)	2 (6)	5 (14)	4 (80)
Maternal fatigue	5 (14)	0 (0)	5 (14)	3 (60)
Persistent occiput posterior presentation	4 (11)	0 (0)	4 (11)	1 (25)
Outlet obstruction	1 (3)	1 (3)	2 (6)	2 (100)
Elective	1 (3)	0 (0)	1 (3)	1 (100)
Failed forceps delivery	0 (0)	1 (3)	1 (3)	1 (100)
Unknown	1 (3)	0 (0)	1 (3)	–
Total	25 (71)	10 (29)	35 (100)	23 (66)

increased after application of the SOVC in four (11%) of the women, but three of the four cases of infection of the genitourinary tract occurred following cesarean section.

Of the 35 infants 12 (34%) had macrosomia[14] and 5 (14%) had shoulder dystocia. Cephalohematoma developed in eight (23%), 75% of whom also had neonatal jaundice; in six of the eight infants the cephalohematoma resolved within the first 6 weeks.

The Apgar score was depressed in nine (26%) of the 35 infants at 1 minute but in only one (3%) at 5 minutes. The 11 infants with fetal distress demonstrated a similar pattern in their Apgar scores; however, 54% had neonatal jaundice. When they were assessed at 6 weeks of age, 10 of the 11 infants (1 was lost to follow-up) were developing normally.

Severe asphyxia at the time of birth occurred in 1 of the 35 infants. There had been spontaneous onset of labour in the 16-year-old primigravid mother at 42 weeks' gestation. After application of the SOVC because of a prolonged second stage of labour, vacuum extraction failed. Delivery with Simpson's forceps also failed. Fetal distress was not diagnosed until the time of delivery by cesarean section. The male infant, who had an Apgar score of 2 at 1 minute and 3 at 5 minutes, required tracheal intubation and was subsequently transferred to the regional neonatal care facility. Follow-up at 24 weeks, by a pediatrician from the Provincial Perinatal Program of Newfoundland, showed the child to be developing normally.

Of all 231 infants born during the study period 2 (0.9%) were stillborn; however, neither death was associated with the use of the SOVC. The perinatal mortality for all of Newfoundland at this time was 9.8%, compared with the national perinatal mortality of 10.8%.[15]

Discussion

Despite careful antenatal screening and referral, 26% of the women followed through labour at the BVPHC during the study period unexpectedly required operative delivery.

Vacuum extraction with the SOVC was a useful adjunct to our obstetric practice, especially in the presence of fetal distress. However, as others have reported,[16-18] its effectiveness in correcting malrotation and promoting descent was enhanced by operator experience. When an experienced physician attributed failure of vacuum extraction to cephalopelvic disproportion, a "trial of forceps"[19] would not be done, and cesarean section would be expedited. This practice is accepted in other centres.[11]

The incidence of cephalohematoma in our study (23%) is comparable to that reported in studies of vacuum extraction with a device having a rigid cup.[20] Except for neonatal jaundice, we found no significant sequelae in our infants. Although shoulder dystocia developed frequently after delivery with the SOVC, there was no subsequent abnormality. Midpelvic delivery is known to be associated with shoulder dystocia, and several authors have reported an increased incidence of this disorder following vacuum extraction.[21,22] We did not determine the incidence of retinal hemorrhage; although this condition has been associated with vacuum extraction with the Malmström device, its clinical significance is uncertain.[21]

As we expected, instrumental delivery seemed to increase the maternal morbidity. However, the rate of cesarean section appeared to decrease after the introduction of the SOVC to our centre. Although the rate of primary cesarean section fell by only 2% from 14% in the 18 months before the study period, the incidence of cephalopelvic disproportion or failure of labour to progress as an indication for cesarean section dropped from 65% to 52%. The use of Simpson's forceps declined in the same period, from 7% to 4%, and the SOVC replaced Kielland's forceps. Although these three instruments have not been compared in controlled studies, vacuum extraction with the Malmström device (introduced in 1954) has been shown to be as safe as or safer than forceps delivery.[5,24-27]

After developing competence in vacuum extraction, physicians may need to reassess the need for the use of forceps and surgical intervention in certain cases. Training in the use of vacuum extraction with an instrument such as the SOVC should be encouraged for the many family physicians practising obstetrics in small rural hospitals.

We are indebted to Dr. G.H. Burgess and the staff of the Baie Verte Peninsula Health Centre and Dr. Y.K. Jeon and the staff of Brookfield Hospital for their assistance, and to Dr. F.R. Papsin for his encouragement.

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Acute nonlymphocytic leukemia following bladder instillations with thiotepa

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A case of therapy-related leukemia is described. Other cases of acute nonlymphocytic leukemia have been associated with the intramuscular administration of thiotepa (an alkylating agent), but this patient received only intravesical instillations of the drug. The interval between the start of chemotherapy and the onset of leukemia was 56 months.

Un cas de leucémie consécutif à une chimiothérapie est décrit. D'autres cas de leucémie non-lymphocytaire aiguë ont été rattachés à l'administration de thiotépa (un agent alkylant) par voie intramusculaire, mais dans le cas qui nous occupe le patient n'avait reçu le médicament que par instillation intravésicale. L'intervalle écoulé entre le début de la chimiothérapie et l'apparition de la leucémie était de 56 mois.

The association between treatment with cytotoxic drugs and the subsequent development of acute leukemia is now well recognized. Like other alkylating agents, thiotepa has been implicated, though usually after intramuscular administration. Transient cytopenia occurs commonly as a result of intravesical instillation of thiotepa, but we report a case in which this form of thiotepa administration was followed by acute nonlymphocytic leukemia.

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Case report

In 1955, at the age of 56 years, the patient first presented with hematuria. He was found to have a moderately large papillary tumour of the bladder. A transitional cell papilloma (grade 1) was resected.

He was seen regularly for follow-up assessment and required further resection or cautery for small recurrences on 10 occasions between 1960 and 1977. The histologic character of these tumours was the same as that of the original neoplasm. In 1976 transurethral prostatectomy was performed. Histologic examination of the prostatic tissue showed nodular hyperplasia.

In 1978 the patient was found to have many small, benign-looking papillary lesions in the bladder. Because of their multifocal nature thiotepa, 60 mg in 60 ml of normal saline solution, was instilled each month from March 1978 until May 1981.

Routine peripheral blood counts remained essentially normal until June 1980, when the platelet count was 111 × 10⁹/l, the leukocyte count 3.3 × 10⁹/l (42% polymorphonuclear leukocytes, 42% lymphocytes, 9% monocytes, 4% eosinophils and 3% band forms) and the hemoglobin concentration 118 g/l. During the rest of the period when thiotepa was being administered the platelet count fluctuated between 100 and 136 × 10⁹/l except for one occasion, when treatment had been deferred for 1 month, and the count reached 150 × 10⁹/l. The leukocyte count varied between 2.8 and 4.8 × 10⁹/l during this time.

Almost a year after thiotepa had been discontinued the patient was admitted to hospital with an apparent viral illness; he was found to be pancytopenic. The hemoglobin level was 80 g/l, the leukocyte count 2.8 × 10⁹/l (with 50% neutrophils) and the platelet count 34 × 10⁹/l. Occasional blast cells were seen in smears of the peripheral blood. A bone marrow aspirate showed a normocellular marrow with erythroid hyperplasia. There was some evidence of dyserythropoiesis, and 5% of the cells were blast cells.

The patient was readmitted after another 6 months because of epistaxis and weakness. He was free of urinary symptoms. His hemoglobin level was 65 g/l, leukocyte count 1.6 × 10⁹/l (52% lymphocytes, 32% polymorphonuclear cells, 9% blast cells, 5% monocytes and 2% basophils) and platelet count 10 × 10⁹/l. Occasional nucleated erythrocytes were seen. The marrow aspirate was hypocellular but showed dysplastic erythropoiesis; 10% of the cells were blast cells. A trephine biopsy specimen was markedly hypocellular,

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01186698 SUPPLIER NUMBER: 07701953 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Protecting abortion rights in the courts.
1989
WORD COUNT: 674 LINE COUNT: 00059

DESCRIPTORS: National Organization for Women--Cases; Operation Rescue--Cases; Abortion--Laws, regulations, etc.; Fetal tissues--Moral and ethical aspects
FILE SEGMENT: HI File 149

27/8/12 (Item 12 from file: 149)
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01183119 SUPPLIER NUMBER: 07358891 (USE FORMAT 7 OR 9 FOR FULL TEXT)
A world without Roe: how different would it be? (abortion law)
1989
WORD COUNT: 1382 LINE COUNT: 00129

DESCRIPTORS: Abortion--Laws, regulations, etc.; Women's rights--Laws, regulations, etc.; Pro-life movement--Laws, regulations, etc.
FILE SEGMENT: MI File 47

27/8/13 (Item 13 from file: 149)
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01151675 SUPPLIER NUMBER: 07396333 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Genug ist genug: a fetus is not a kidney. (fetal tissue transplantation)
1988
WORD COUNT: 6056 LINE COUNT: 00580

DESCRIPTORS: Abortion--Analysis; Fetal tissue transplantation--Moral and ethical aspects
STATUTE NAME: Uniform Anatomical Gift Act--Interpretation and construction
FILE SEGMENT: HI File 149

27/8/14 (Item 14 from file: 149)
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01151674 SUPPLIER NUMBER: 07396265 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Rights, symbolism, and public policy in fetal tissue transplants.
1988
WORD COUNT: 7017 LINE COUNT: 00670

DESCRIPTORS: Abortion--Analysis; Fetal tissue transplantation--Moral and ethical aspects; Medical research--Laws, regulations, etc.; Bioethics--Analysis
STATUTE NAME: Uniform Anatomical Gift Act--Interpretation and construction

ULTRASONIC LIVER SURGERY

The course will be offered in Ann Arbor, Mich., on June 2.
Contact Betty Phillips, CME Office, G-1100 Towsley Ctr.-Box 0201, Univ. of Michigan Medical School, Ann Arbor, MI 48109-0201; or call (313) 763-1400.

FAMILY PRACTICE REFRESHER AND BOARD REVIEW

The 16th annual course will be offered in Santa Monica, Calif., May 30–June 3.
Contact University of California, Los Angeles, Extension, Health Sciences Dept., Los Angeles, CA 90024; or call (213) 825-7527.

TEACHING MEDICAL INTERVIEWING

The faculty development course will be offered in Miami, June 12–16.
Contact Dr. Mark Multach, Univ. of Miami School of Med., P.O. Box 016960 (R-103), Miami, FL 33101; or call (305) 547-6134.

GASTROENTEROLOGY

The course, subtitled "Recent Developments in Theory and Practice," will be offered in San Francisco, June 14–16.
Contact Extended Programs in Medical Educ., Registration Office, 533 Parnassus, Box 0766, San Francisco, CA 94143-0766; or call (415) 476-5208.

EPIDEMIOLOGY

The graduate summer program will be held in Baltimore, June 19–July 7. Applications must be received by June 1.
Contact Helen Walters, Dept. of Epidemiology, Johns Hopkins Univ., School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, MD 21205; or call (301) 955-3462.

GEORGETOWN UNIVERSITY MEDICAL CENTER

The following courses will be offered in Washington, D.C.: "Wound Management" (June 3); "Lymphomas: Current Concepts in Pathogenesis and Management" (Sept. 21–23); and "3rd International Symposium on Hepatitis Delta Virus" (Oct. 26–28).
Contact CME Office, Georgetown Univ. Medical Ctr., 3800 Reservoir Rd., NW, Washington, DC 20007; or call (202) 687-8735.

AMERICAN DIABETES ASSOCIATION

The 49th annual meeting and scientific sessions will be held in Detroit, June 1–6.
Contact Christina Peppas, ADA, Meetings Dept., 1660 Duke St., Alexandria, VA 22314; or call (703) 549-1500.

OCCUPATIONAL AND ENVIRONMENTAL MEDICINE

The 8th annual symposium with postgraduate mini-courses will be held in Sacramento, Calif., June 1 and 2.
Contact Nina Musselman, CME Office, University of California, Davis, School of Med., 2701 Stockton Blvd., Sacramento, CA 95817; or call (916) 453-5390.

SPECIAL REPORT

THE ETHICAL USE OF HUMAN FETAL TISSUE IN MEDICINE

In the past few years, medical science has made dramatic advances through research that uses tissue from dead human fetuses. At the same time, this research has led to controversy over the ethics of using human fetal tissue, particularly tissue from induced abortions. In the spring of 1988, the Department of Health and Human Services halted all therapeutic research using tissue from fetuses aborted electively — stopping first all research at the National Institutes of Health and then any research by the Public Health Service — until the ethical considerations it involves have been studied further.[1,2]

This report, like the department's moratoriums, deals with tissue taken from dead human fetuses. Our discussion is set within four generally accepted principles of medical ethics: beneficence, doing no harm, respect for the autonomy of the patient, and respect for human dignity.[3] The principles do not lead to easy answers in this area. An induced abortion harms the fetus while it respects the patient's autonomy; the subsequent medical use of tissue from that abortion may be beneficent in preserving and improving the lives of others. We conclude, however, that subject to certain important conditions, ethical considerations allow the appropriate medical use of human fetal tissue.

Human fetal tissue has been a valuable research tool since the 1930s, as a source of human cell lines. These cell lines have been widely used in research on viruses and the preparation of vaccines — notably the polio vaccine — against them. More recently, research has been conducted on the transplantation of fetal tissue into living subjects for therapeutic purposes.[4] Three properties make human fetal tissue particularly useful in transplants: it grows rapidly, it is very adaptable, and when it is treated properly it evokes little or no immune response from the host.[4]

Because of these properties, transplants of human fetal tissue have been used experimentally in the treatment of several conditions, such as Parkinson's disease, diabetes, and radiation-induced anemia.[4] Transplants of fetal tissue have been used in more fundamental research to implant a human immune system in immunosuppressed mice, with great potential importance for research into the human immune system and its disorders.[5] These uses remain experimental, but they and many others offer the hope of new medical treatments for millions of people. The benefits they may bring cannot be guaranteed, but the opportunities to preserve life and alleviate suffering could be enormous.

The use of human fetal tissue is currently subject to several legal constraints. Federal regulations govern its use in federally funded research.[6] They require that the research be separated from the performance of the abortion, and they prohibit any inducements, monetary or otherwise, to the mother for the purposes of performing the research.[7] Beyond this, they require only that applicable state and local law be followed.[8] All 50 states and the District of Columbia have adopted the Uniform Anatomical Gift Act, which expressly applies to fetal tissue.[9] (In 1987 a revised version of the act was adopted by the Commissioners on Uniform State Laws, but the new version has not yet been adopted by any states.[10]) Many states have adopted additional legislation on the disposition of fetal re-

mains and their use in research[11,12]; a few states, such as Arizona, have banned all such use,[13] although not without raising serious constitutional issues.[14]

The Use of Human Fetal Tissue Is Not Itself Objectionable

Although fetal tissue has distinctive biologic properties as compared with other human tissue, it does not in itself have distinctive ethical properties. Considered without regard to the cause of fetal death, tissue from a deceased human fetus is entitled to the same treatment and respect as tissue from a deceased child or adult, neither more nor less. In transplantation, for example, tissue from a fetus that spontaneously aborted should be treated with the same respect and subject to the same rules as tissue from the cadaver of an accident victim.

Transplantation is governed by the Uniform Anatomical Gift Act, which in the case of a fetus provides that either parent may donate the fetal remains as long as the other parent does not object.[15] The act further provides that the physician certifying death shall not participate in the removal or transplantation of any body parts.[16] State law and medical ethics impose additional constraints. The remains should be treated with respect and any tissue not used should be disposed of properly. Such respect should require that human fetal tissue be used only for matters of medical benefit — medical treatment, research, or education.

The only relevant difference between fetal tissue from a spontaneous abortion and tissue from the cadaver of an adult is that the fetus was never in a position to choose whether its cadaver should be used. The Uniform Anatomical Gift Act does not require that decedents should have given consent for the medical use of their cadavers. If a decedent has expressed no preferences for or against medical use, the next of kin as defined by the act can make the decision. When an abortion is spontaneous, the fact that the decedent is a fetus does not change the ethical considerations behind that decision.

Applying to the use of human fetal tissue the legal and ethical constraints that govern the use of cadavers affirms that fetuses have moral value. Other kinds of tissue removed from an adult, such as an infected appendix or a benign tumor, do not raise the same issues. A fetus is not simply a mass of tissues; it is at least a potential human life and should be treated with dignity.

The Ethical Use of Fetal Tissue from Induced Abortions

The use of human fetal tissue is not in itself ethically objectionable when handled under the conditions we have set out. Fetal tissue differs from the tissue of adult cadavers, however, because it often becomes available as the result of a mother's decision, implemented by a physician's actions, to end her pregnancy. This element of volition introduces a new ethical problem. It raises the possibility that the subsequent use of fetal tissue will encourage abortions that would not otherwise have occurred. If tissue from spontaneous abortions could reasonably satisfy medical demands in both quantity and quality, it would be preferable to avoid the ethical problems of using tissue from induced abortions. Subject to certain additional constraints, however, fetal tissue acquired as a result of induced abortions can ethically be used.

Whether or not induced abortions should be legal, ending a pregnancy through an abortion is not an affirmatively "good" thing. The ethical principles of preserving life (or potential life) and doing no harm dictate that the use of fetal tissue should not be allowed to encourage abortions that would not otherwise take place. Similarly, conception should not be undertaken for a purpose other than bearing children. The use of fetal tissue must not be allowed to encourage pregnancies that would not otherwise occur. These points have two important consequences.

First, patients should not be allowed to derive any benefit from the use of tissue from fetuses they have elected to abort. To allow otherwise would raise all the ethical problems of organ selling, with the additional problem of paying someone for preventing or ending a life. Federal regulations currently prohibit federally funded research projects from offering women "inducements" to participate in fetal-tissue research. More generally, the National Organ Transplantation Act prohibits any sale, purchase, or brokerage of certain human organs for transplantation, but these do not include fetal tissue.[17] (The revised Uniform Anatomical Gift Act contains a similar ban on the commercialization of human organs.[18])

These legal constraints should be expanded. The National Organ Transplantation Act's prohibition of organ transactions should be extended to include the direct use of fetal tissue. The act should then be amended to exclude from the reimbursement it permits any expenses associated with induced abortions. Whatever one's position on access to abortions, providing free abortions only on the condition that the fetal tissue be used for medical purposes is unjustifiable. The act should also be extended beyond transplantation and the federal regulations extended beyond research to reach all medical uses of human fetal tissue.

Second, patients are not the only parties whose possible incentives can raise ethical problems. Those performing abortions should not be allowed to benefit from the subsequent use of the fetal tissue. Physicians and other medical personnel may have great influence over their patients' decisions concerning abortion. If they have direct personal interests in the resulting fetal tissue, they may, consciously or not, encourage their patients to have abortions.

Under current federal regulations, physicians who perform induced abortions cannot participate in federally funded research that uses tissue from these abortions. To avoid the ethical problems of conflicting interests, this limit should be broadened. Medical personnel who participate in an abortion should not receive any direct benefit from the subsequent use of

fetal tissue from that abortion. Everything from direct payments to the special availability of fetal tissue to their patients should be prohibited. If the tissue is to be used within the institution where the abortion is performed, the institution should neither reward the doctors performing the abortion for providing it nor discipline them for failing to provide it.

The Ethical Use of Fetal Tissue in Three Specific Situations

Induced abortions remain intensely controversial in the United States. In the application of the general principles we have discussed, three types of induced abortions must be distinguished: those induced because of risk to the woman's life, those induced for the purpose of obtaining fetal tissue, and those induced for other purposes.

Abortions Induced because of Risks to the Woman's Life

Inducing abortion when a woman's life is at risk from the continuation of her pregnancy is widely approved. Before *Roe* v. *Wade*, state law generally allowed abortion when the mother's life was threatened. Today, the federal government continues to assist in financing such abortions for women who cannot afford them, and few political opponents of abortion object to them. Under the doctrine of double effect, usually attributed to St. Thomas Aquinas,[19] even the Roman Catholic Church permits "indirect" abortions in some life-threatening situations, such as ectopic pregnancy and cancer of the uterus.[20] It seems very unlikely that the possible use of the fetal tissue will strongly influence women facing a risk of death in their decision on abortion. These abortions are ethically unobjectionable to almost everyone. The use of fetal tissue from them raises no more issues than does the use of tissue from spontaneously aborted fetuses.

Abortions Induced for the Purpose of Contributing Fetal Tissue

According to several widely circulated reports, women have offered to have abortions in order to provide fetal tissue for specific recipients.[21,22] Physicians and medical centers should not use the human fetal tissue from such abortions. To use that tissue is to treat the fetus as nothing but a medical product and the uterus as a factory. It would demean the potential or actual humanity of the fetus.

Under existing regulations, the designation of a recipient for the tissue from such an abortion might be barred in federally funded research as constituting an inducement to a woman to participate in the research. The Uniform Anatomical Gift Act, however, allows organ donations to specific persons for medical purposes.[23] The act should be amended to bar the designation of a recipient of fetal tissue.[24] Under existing law, a woman may have an abortion for any purpose or none at all, but physicians and medical centers should not encourage pregnancies and abortions that would not otherwise have occurred, by using designated donations.

This problem of designated recipients does not arise with spontaneous abortions, because the possibility of designation does not lead to the abortion. Current law allows designated donations of tissue from children and adults, although there is continuing debate about the justice of the practice. The ethics of designated donations of tissue from clearly spontaneous abortions should be treated as part of that debate; the fetal source of the tissue raises no special questions when the abortion is not intentional.

Abortions Induced for Other Purposes

With respect to other abortions, the crucial ethical issue is whether the medical use of fetal tissue encourages induced abortions. If it does not, the propriety of the abortion itself seems irrelevant to the subsequent use of the fetal tissue.

In general, the cause of a death that makes organs or tissue available for medical use should be irrelevant as long as the potential medical use did not contribute to the death. We as a society have already decided that the medical use of cadavers is not inherently disrespectful to the deceased. Indeed, beneficial use of the cadaver allows some good to be salvaged from a death.

The conditions we propose for the ethical use of fetal tissue try to ensure that the subsequent use of tissue from an induced abortion provides no benefit to the woman undergoing it or to those performing it. It is possible of course that even with these restrictions, the mere knowledge that a beneficial use may be made of the fetal tissue could influence some women considering abortions. In the light of the deeply personal and powerful physical, emotional, economic, and religious concerns of women considering abortions, it seems implausible that this knowledge would have any marked effect.

Not all physicians and medical centers will draw the same conclusions. Some may feel that the incentive created by a beneficial use of aborted fetal tissue would have a substantial effect on women contemplating abortions. Others may believe that abortion, although legal, is so wrong that they should take no part in it under any circumstances. In the absence of empirical evidence, the first argument cannot be definitely resolved; by its nature, the second argument can never be definitively resolved. Given these legitimate differences, a physician or medical center should never feel ethically obligated to participate in research or therapy involving human fetal tissue.

Our discussion has drawn a sharp distinction between the use of fetal tissue from spontaneous abortions and from induced abortions. Our concern is to prevent the subsequent use of the tissue from encouraging induced abortions. It is possible that this distinction would lead women and physicians to break the law by pretending that abortions are spontaneous when they are in fact induced. Any law can be broken, but physicians performing abortions and physicians using human fetal tissue seem unlikely to create together a criminal black market. If future experience under our suggested guidelines showed that disguised

induced abortions were a great problem, we would seriously consider extending the guidelines to spontaneous abortions as well.

The Proposals

The issue of induced abortion bitterly divides many in our society. The emotional depth of that division necessarily affects the medical use of fetal tissue from induced abortions. A careful analysis of the relation between fetal tissue and abortion, however, reveals useful ground that all or almost all can share and leads to the proposals that follow.

Human fetal tissue should generally be treated with the respect given cadavers, and its use should be governed by the same legal rules.

Women who undergo induced abortions should not be allowed to benefit directly from the subsequent medical use of the fetal tissue, through payment for it, through the reimbursement of expenses connected with the abortion, or in any other manner. The National Organ Transplantation Act should be amended to cover human fetal tissue — whether used for transplantation or any other medical purpose — and to exclude abortion-related expenses from its definition of permissible reimbursement.

Because of the possibility that a conflict of interest might affect their advice to patients about abortion, medical personnel who perform induced abortions should not be allowed any direct benefit from the subsequent use of the fetal tissue.

The proper medical use of fetal tissue from spontaneous abortions and from abortions induced because of risk to the mother's life is ethically unobjectionable.

The use of tissue from fetuses aborted for the specific purpose of donating that tissue seems ethically impermissible. The Uniform Anatomical Gift Act should be amended to bar the donation to specific persons of human fetal tissue from induced abortions.

Subject to these conditions, human fetal tissue can be used ethically for medical research and treatment.

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The members of the Stanford University Medical Center Committee on Ethics were as follows: Diana Akiyama, associate dean, Memorial Church; Ronald Ariagno, M.D., professor (clinical) of pediatrics; Carol Bernhardt, community representative; Helen Blau, Ph.D., associate professor of pharmacology; Cynthia Cannady, J.D., community representative*; Ari Carton, director, B'nai B'rith Hillel Foundation; Ruth Crooklin, Ph.D., research health science specialist, Palo Alto Veterans Administration Medical Center and senior lecturer in sociology; Lawrence Crowley, M.D., professor of surgery; Julian Davidson, Ph.D., professor of physiology; Leslie Dorfman, M.D., associate professor of neurology; John Dupre, Ph.D., assistant professor of philosophy; Brad Dyer, medical student; A.T. Ganesan, Ph.D., professor of genetics; Michael Gillis, J.D., community representative; Henry Greely, J.D., associate professor of law; George Gulevich, M.D., professor of psychiatry; Thomas Hamm, D.V.M., Ph.D., director of the Division of Laboratory Animal Medicine; Peter Hammond, Ph.D., professor of economics; Timothy Jackson, Ph.D., assistant professor of religious studies*; Rodney Johnson, J.D., managing attorney for medical affairs; Richard Kelly, medical student; Jeanne Kennedy, director of community and patient relations, Stanford University Hospital; Sheldon King, president, Stanford University Hospital; David Korn, M.D., vice president and dean, School of Medicine; James Mark, M.D., chief of staff, Stanford University Hospital; David Maurice, Ph.D., professor (research) of surgery; Margaret McAfee, dean's office staffperson, School of Medicine; Bruce McAuley, M.D., medical staff (internal medicine), Stanford University Hospital†; Mary Miller, director of publications, Stanford University Hospital; Joyce Miller, R.N., assistant director of nursing, Stanford University Hospital; Nicholas Ney, Ph.D., Alcohol and Drug Treatment Center staffperson; Carole Price, J.D., director of physician services and risk management, Stanford University Hospital; Donna Pronko, M.P.H., patient relations coordinator, Stanford University Hospital; Thomas Raffin, M.D., associate professor and assistant chief of medicine (chairperson, Stanford University Medical Center Committee on Ethics); John Ruark, M.D., medical staff (psychiatry), Stanford University Hospital; Michael Stephanides, medical student; Susan Stevens, M.D., resident in diagnostic radiology; David Stevenson, M.D., associate professor of pediatrics; Edward Stinson, M.D., professor of cardiovascular surgery†; James Thomas, medical student; Kent Ueland, M.D., professor and chairman, gynecology and obstetrics; Vera Waechter, R.N., director of clinic nursing; Randy Weingarten, M.D., medical staff (psychiatry), Stanford University Hospital; Lewis Wexler, M.D., professor of radiology; Robinetta Wheeler, Ph.D., R.N., clinical nursing coordinator; Charles Whitcher, M.D., professor (clinical) of anesthesia; Emie Young, Ph.D., chaplain to the medical school; and James Zehnder, M.D., chief resident in internal medicine.

The results of the committee's vote were as follows: approve, 44; disapproves (*), 2; abstain (†), 2.

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5/3,K/1 (Item 1 from file: 5)
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Utility of fragmented human fetal tissue as a potential dopaminergic brain graft in Parkinson's disease.
AUTHOR: Hogenesch R I(a); Staal M J; Kema I P; Buys C H C M; Go K G
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JOURNAL: Stereotactic and Functional Neurosurgery 61 (1):p1-11
ISSN: 1011-6125
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LANGUAGE: English

ABSTRACT: There is increasing interest in the use of human **fetal** dopaminergic **tissue** as a source of striatal **transplant** in parkinsonian patients. This tissue is acquired by elective abortions. The possibilities of the use...

...by high performance liquid chromatography. It turned out that 50% of the curettages obtained by **suction** abortion were too fragmented to reliably recognize the dopamine-containing area (ventral mesencephalon). Furthermore, dissection...

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5/3,K/2 (Item 2 from file: 5)
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CRYOPRESERVATION OF HUMAN BRAIN TISSUE
AUTHOR: ROBBINS R J; TORRES-ALEMAN I; LERANTH C; BRADBERRY C W; DEUTCH A Y; WELSH S; ROTH R H; SPENCER D; REDMOND D E JR; NAFTOLIN F
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JOURNAL: EXP NEUROL 107 (3). 1990. 208-213. 1990
FULL JOURNAL NAME: Experimental Neurology
CODEN: EXNEA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

...ABSTRACT: products of conception were examined to determine the feasbility of obtaining viable neural tissue after **suction** abortion at 9-12 weeks of gestation. The ventral mesencephalon, a prototype region whose maturation can be monitored and which is a potential tissue for **transplantation** , was identified in 32 of 120 cases. The tissue was then screened for the presence...

...vitro exhibited neuronal morphology, tyrosine hydroxylase immunoreactivity, and dopamine production. We have demonstrated that human **fetal** brain **tissue** can be cryopreserved in a manner which not only retains viability but allows normal phenotypic...

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05762247 BIOSIS NO.: 000084110654
FETAL TECTAL OR CORTICAL TISSUE TRANSPLANTED INTO BRACHIAL LESION CAVITIES IN RATS INFLUENCE ON THE REGROWTH OF HOST RETINAL AXONS
AUTHOR: HARVEY A R; GAN S K; PAUKEN J M
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JOURNAL: J COMP NEUROL 263 (1). 1987. 126-136. 1987

EXPERIMENTAL NEUROLOGY 107, 208–213 (1990)

Cryopreservation of Human Brain Tissue

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Tissues from products of conception were examined to determine the feasibility of obtaining viable neural tissue after suction abortion at 9–12 weeks of gestation. The ventral mesencephalon, a prototype region whose maturation can be monitored and which is a potential tissue for transplantation, was identified in 32 of 120 cases. The tissue was then screened for the presence of infectious agents, while being held at −196°C in cryopreservative solutions. Three of 32 specimens were found to be contaminated by normal vaginal bacteria; all other viral, fungal, and mycoplasma testing was negative. Thawed brain fragments retained high viability after storage in liquid nitrogen and when grown *in vitro* exhibited neuronal morphology, tyrosine hydroxylase immunoreactivity, and dopamine production. We have demonstrated that human fetal brain tissue can be cryopreserved in a manner which not only retains viability but allows normal phenotypic differentiation after thawing. © 1990 Academic Press, Inc.

INTRODUCTION

Restoration of complex neurologic functions by transplantation of brain cells into rodents and primates (3, 4, 8) has generated interest in similar approaches for treating neurologic diseases in man. Human disease states in which specific neurochemical deficits exist, such as the neostriatal dopamine deficiency in parkinsonism, seem to be an appropriate starting point at which to attempt to apply such methodology. Reversal of the clinical manifestations of parkinsonism by medical therapy which increases central dopaminergic tone has been clearly documented (21). Evidence from dopamine-deficient rodent and primate models suggests that transplanted fetal dopamine-producing cells can also rectify the neurologic impairments (5, 6, 12). Improvements have also been reported after transplantation of different brain regions into animal models of Alzheimer's disease (3), Huntington's disease (8), and cortical blindness (17).

The possibility of transplantation of human fetal neural tissue is a logical extension of similar work in animal models (14, 19). Apart from the complex scientific and ethical (20) issues raised by such an approach, many practical problems need to be addressed regarding the safety, identity, and viability of the tissue, and the means by which the tissue could be preserved while safety testing was proceeding.

We have begun to resolve several of these questions using, as a prototype, ventral mesencephalic tissue collected from human fetal cadavers soon after death. Since brain tissue from rats (7, 9) and monkeys (1) retains viability and function during cryopreservation we assessed the utility of this method for preserving and holding human fetal cells. While the tissue was cryopreserved screening tests for the presence of infectious agents were carried out on adjacent brain tissue from the same fetus. We found that after thawing, cryopreserved mesencephalic cells retain high viability, express tyrosine hydroxylase, and synthesize dopamine.

METHODS

Tissue collection. Prior to scheduled abortion of normal first trimester (7–12 weeks old) fetuses, permission was obtained from the gravida to study cadaver tissues and cells according to a protocol approved by the Human Investigation Committee of Yale University School of Medicine. The ethical guidelines for this protocol have been previously described (14). Following vaginal preparation with Betadine sponges, dilation of the cervix was performed under local anesthesia. A number 10–12 cannula was used to evacuate the uterus. The sterile aspiration bottles containing the products of conception were brought to a tissue culture laboratory where they were emptied onto an autoclaved stainless steel screen and rinsed with iced, sterile 0.01 *M* phosphate-buffered saline (PBS). Midbrain fragments were identified by gross morphology and were then dissected with the aid of a dissecting microscope by one of us (C.L.) to obtain 1- to 2.5-mm^3 pieces from the region of the substantia nigra (SN) and the ventral tegmental area (VTA). This area was identified by surface landmarks: the crus cerebri and the interpeduncular fossa (IF). Tissue from the middle third of the ventral mesencephalon rostral to the IF was collected. The tissue was held at 4°C in sterile test tubes containing PBS with 5 m*M* glucose and bubbled with carbogen to maintain a high oxygen concentration.

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Time from the end of the suction procedure to arrival of the tissue in the laboratory was 3–5 min; the collection and dissection of fragments required from 2 to 8 min. Time after collection of SN/VTA fragments on ice until freezing varied from 30 to 180 min. Viability of the cells, judged by trypan blue exclusion, did not vary within these time intervals.

Freezing and storage. Tissue blocks were prepared for freezing by exposing them to increasing concentrations of dimethyl sulfoxide (DMSO) in Hanks' balanced salt solution (HBSS) at 4°C in the dark. Ten-minute incubations in 0.25, 0.5, 1.0, and 1.5 *M* DMSO were performed. Blocks were then aspirated into freezing straws (IMV, Minneapolis, MN) and cooled to −6°C. Ice nucleation was begun away from the tissue, which was then held at −6°C for 30 min, after which the tissue was cooled in a controlled rate freezer (Planer Freezer Model R 204, TS Scientific) at a rate of 0.3°C/min until −80°C at which point the straws were rapidly immersed into liquid nitrogen. Some tissue blocks were frozen using propanediol as the cryopreservative according to the method of Testart and colleagues (18). Viability, as determined above, was always greater than 95% using this cryopreservation technique. When plated as described below, the propanediol-preserved cells grew as well as those preserved in DMSO.

Cell culture. Frozen tissue blocks in straws were rapidly thawed by immersion into a 37°C bath under sterile conditions. DMSO was cleared by repetitive rinses in culture medium (see below). The blocks were then incubated in a papain–neutral protease–DNAse solution (10) in HBSS and dispersed by gentle trituration every 15 min for 45 min at 37°C. The cells were pelleted by low speed centrifugation, resuspended in culture medium (DMEM–Ham's F-12 (1:1) with 20% fetal calf serum (FCS), and counted in a hemocytometer. In specimens which stayed completely submerged for 1 week to 3 months in liquid nitrogen, cellular viability was always greater than 90%. Viability was reduced (as low as 77%) in DMSO-cryopreserved tissues which had been repeatedly removed and replaced in the liquid nitrogen during the retrieval of other specimens. Cells were plated at 4–5 × 10^5 cells/35 mm^2 dish in 2 ml of culture medium. Dishes or glass coverslips were coated with gelatin, poly-L-lysine, and FCS. The medium was changed every fourth day.

Immunohistochemistry. Seven-day-old SN/VTA cultures were examined for the presence of tyrosine hydroxylase-like immunoreactivity (THLI). Cells from the caudal brain stem (not including the SN/VTA) were cultured as controls. Cultures were rinsed in PBS at 4°C, fixed by immersion in 4% paraformaldehyde for 30 min at 25°C, and stained for THLI as previously described (2). The presence of THLI in freshly thawed SN/VTA blocks was examined. Three blocks, each from a different source, were thawed, immediately fixed with 4% paraformaldehyde, stained for THLI, as above, and flattened between a microscope slide and a coverslip ("squash-prep"). The presence of THLI was then determined by light microscopy.

Dopamine assay. Tissue dopamine content was determined as follows: Cells were collected and homogenized in extraction buffer (500 μl concd $HClO_4$ and 500 μl 10% sodium metabisulfite were diluted up to 50 ml with distilled water; dihydroxybenzylamine (DHBA) was added as an internal standard, to a final concentration of 0.5 pg/μl). After centrifugation at 3000*g* for 5 min the supernatant was analyzed for dopamine and the pellet for DNA. A specially designed HPLC chromatographic system was used for analysis of dopamine. Narrow-bore (2.1 mm i.d.) columns were packed with 3 μm C-18 particles (Phase Separations, Norwalk, CT). The mobile phase was 0.2 *M* phosphate buffer containing octanesulfonic acid (200 mg/liter), 15% methanol, and 0.1 m*M* EDTA, *p*H 2.9. A battery-powered potentiostat was used for applying a potential to the glassy carbon detector electrode (Bioanalytical Systems, West Lafayette, IN). This HPLC system, along with more recent refinements (Bradberry and Roth, in preparation) allowed for routine limit of detection of 2–3 fmol; this level of sensitivity was necessary for the detection of dopamine in these samples. An alumina extraction procedure was performed prior to the HPLC analysis (15).

Microbiological testing. When SN/VTA tissue was identified by microdissection, adjacent tissue was transported on ice to the microbiology laboratories where it was homogenized in a tissue grinder and inoculated onto plates containing brain–heart infusion broth, blood agar, chocolate agar, thioglycollate broth, anaerobic blood agar, MacConkey's medium, Mycotrim plates, or Sabauraud's medium. Another adjacent tissue fragment was sent to the Yale Virology Laboratory where it was homogenized and plated in human placenta and foreskin lines, a human cervical carcinoma line (HEP2), a green monkey kidney line (VERO), and a rhesus kidney cell line to screen for a wide variety of viruses, including cytomegalovirus. In addition, in cases in which SN/VTA tissue was recovered, serum from the gravidae was screened for Hepatitis B and for HIV.

RESULTS

Products of conception from 120 pregnancies were examined. An intact midbrain, which allowed dissection of SN/VTA dopamine neurons with a high degree of confidence, was present in 32 cases. We found that an intact midbrain was most likely to be present and to be large enough for dissection of the SN/VTA in fetuses between 9 and 12 weeks of age. Following suction evacuation the midbrain of older fetuses was too disrupted for identifi-

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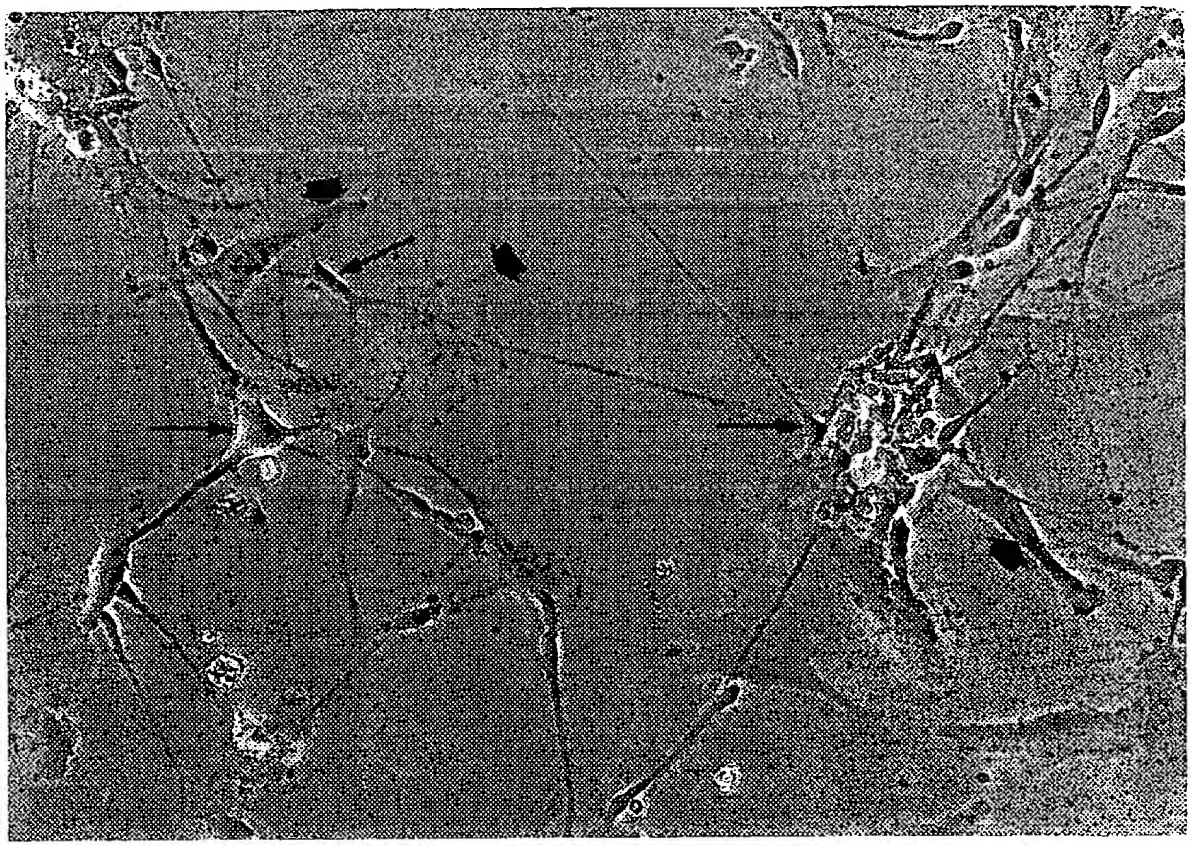

FIG. 1. Phase-contrast photomicrograph at 200× of a 7 DIV culture of human ventral mesencephalic cells in monolayer culture. Both neuronal (thin arrows) and glial (arrowheads) elements are visible.

cation of necessary landmarks. After thawing, tissue blocks of approximately 1 mm^3 yielded between 2.5 and 6×10^5 cells. For comparison an unfrozen 1-mm^3 SN fragment yielded 5×10^5 cells.

When fragments which had been frozen for 10–14 days were thawed, dispersed, and cultured, cells began to attach to the dishes by 2 h. Neurite extension was seen regularly by 5 h after plating. By 24 h, cells with the morphology of neurons were easily distinguishable from glial elements by phase-contrast microscopy. The neurons (Fig. 1) were phase-bright with perikaryal halos. Both fusiform and stellate phenotypes were present with highly branched processes (300–500 μm in length). Initially cultures were highly enriched in neuronal phenotypes; however, by 7 days *in vitro* (DIV) many glial elements had appeared and formed a basal monolayer upon which many large neuronal cells could be seen.

THLI-positive neurons were present in SN/VTA cultures after 7 days *in vitro*. Approximately 1% of all of the neuronal cells *in vitro* stained for THLI (see Fig. 2). These cells had many small dendrites and, in some cases, axons which extended more than five times the diameter of the perikaryon. In most instances, THLI was present throughout the entire length of the processes. By comparison, more than 50% of the immature cells in the "squash-preps" of freshly thawed 9- to 10-week-old SN/VTA blocks ($n = 3$) stained for THLI.

The presence of THLI suggested that the cells might be functional. To directly confirm that dopamine was being produced, three SN/VTA fragments were separately cultured (5×10^5 cells/well; $n = 5$ wells). At 10 DIV, cells from each fragment were separately harvested for measurement of dopamine. Cultures contained 117.9 ± 37 (mean ± SEM) pg dopamine/10^6 cells. The concentration of dopamine in the cells was 18.1 ± 4.2 pg/μg DNA. Cultures ($n = 4$) of brain fragments adjacent to the SN/VTA region were grown in a similar fashion; no dopamine was detected in these cells. For comparison, a 1-mm^3 SN/VTA fragment from a 9-week-old fetus was homogenized prior to cryopreservation and found to contain 9.3 pg of dopamine.

In the 32 cases examined, three tissues exhibited single organism bacterial growth of normal vaginal flora (coagulase-negative Staphylococcus, Bacillus species [not typed], and β Streptococcus group B). When the SN/VTA tissues from two of these cases were thawed and cultured, rapid bacterial overgrowth was found in both, confirming the utility of the bacteriologic screen-

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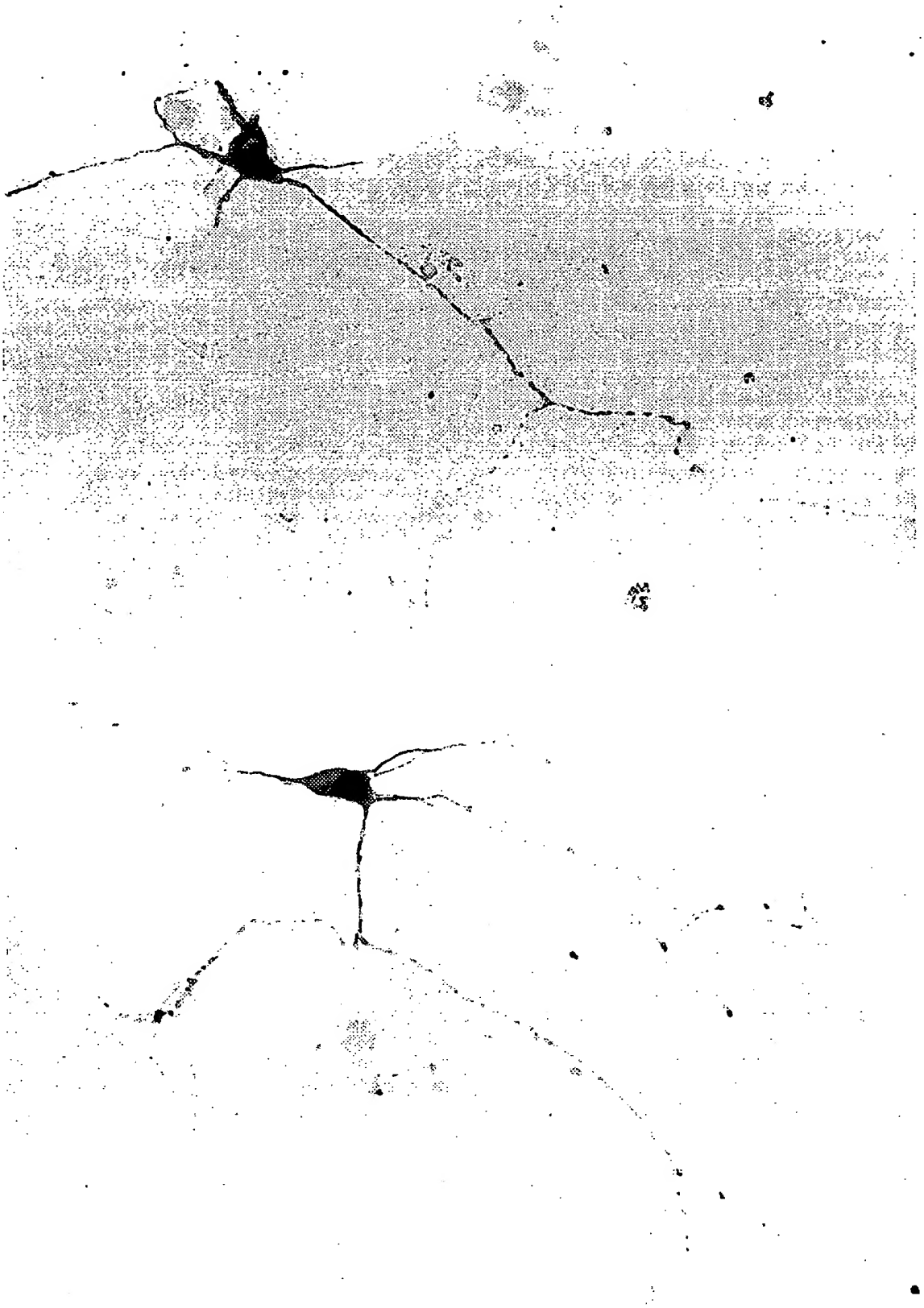

FIG. 2. Photomicrograph of human fetal neurons which were stained for tyrosine hydroxylase-like immunoreactivity (THLI). Very long axonal and dendritic processes are visible in THLI-positive cells after 7 days *in vitro*.

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ing procedure. All viral studies, including HIV, were negative. One patient had positive antibodies against hepatitis B; however, B surface antigen testing was negative.

DISCUSSION

These studies demonstrate that specific brain regions can be aseptically recovered from first trimester human fetal cadavers after suction abortion and, after cryopreservation, remain viable and retain the capacity to extend neurites and to produce region-specific neurochemical markers.

The ventral mesencephalon was chosen for our initial studies because it is a discrete area which can be identified by topographical landmarks. It also has characteristic developmental markers such as TH and dopamine. The SN/VTA is known to be involved in motor control in man, and damage to cells in this region in humans is associated with parkinsonism. Transplantation of this region into the brains of animals with induced dopamine deficiency corrects many of the neurologic defects. We have recently reported that human fetal SN/VTA tissue obtained and cryopreserved using these methods survives and develops when transplanted into monkeys (14).

These studies were undertaken with the rationale that if such tissue were to be utilized for human transplantation it would have to undergo safety testing of several weeks duration. Careful evaluation of potential candidates and surgical preparation of a recipient for the transplant would be optimized if the tissue could be made available to the surgeon at a precise time in a state of high viability. The fact that several tissue fragments (9%) were contaminated with normal vaginal bacteria indicates that microbiologic screening should be an important element of any program intending to use human fetal tissue for transplantation. The value of more stringent vaginal cleansing protocols in preventing such contamination must be established. Although we considered it unlikely that apparently normal fetal brain tissue would contain viral particles, we did screen for a wide range of vaginal and CNS viruses; in no case was a positive culture seen. The viral cultures employed cannot detect the presence of slow viruses. Since our viral cultures are normally held for 6 weeks, this is the minimum waiting period necessary for its potential use as a transplant.

We chose cryopreservation over cell culture as a holding method for several reasons. Cryopreservation preserves the normal cell–cell interactions present in the developing SN/VTA, which would be disrupted during dispersal of the tissue for cell culture. Second, biological processes such as aging proceed at undetectable rates at −196°C (11), whereas maturation and differentiation proceed *in vitro*, potentially limiting the ability to adapt to a transplant environment. Finally, unlike cells in culture, cryopreserved blocks do not become infected while in liquid nitrogen nor do they have to be disrupted again prior to use in transplantation.

The use of either DMSO or propanediol resulted in high levels of viability of the thawed tissue. The immunohistochemical studies indicate that dopaminergic neurons survive cryopreservation while retaining the ability to extend dendrites and axons and to express a neuronal phenotype. The immaturity of this area at 9–12 weeks of age may explain the low tissue concentrations of dopamine. The fact that only 1% of the neuronal cells, after 7 DIV, express THLI as compared to the 50% level in the "squash-preps" prior to culture may represent damage during the tissue dispersal, lack of appropriate neurotrophic factors in the tissue culture medium, or transient expression of TH in a larger number of fetal cells which is lost during differentiation. It is important to point out that the survival *in vitro* in no way predicts the survival *in vivo*, especially with regard to potential viability in parkinsonian striatum.

The presence of dopamine in the SN/VTA cultures and not in cultures of adjacent non-SN/VTA tissue confirms that the correct tissue was collected and that the THLI represents functional TH. The presence of picomolar concentrations of dopamine in the cells after 10 DIV demonstrates the ability of these cells to mature rapidly in a foreign environment after cryopreservation. Studies are underway to examine the ability of tissues held long term (13 months to 2 years) to grow *in vitro* and to produce THLI and dopamine.

We believe that the ability to retrieve precisely, and characterize carefully, human neural tissues will markedly improve the chances for success of cellular replacement therapy in neurodegenerative diseases. However, the potential of human fetal neurons to reverse the neurologic manifestations of a human neurodegenerative disease such as parkinsonism remains speculative. Although fetal SN/VTA tissue can produce dopamine and survive in animals with damage to substantia nigra (13), there are certainly other cellular and humoral elements in these tissue blocks which contribute important factors for the development of dopamine neurons and which may provide signals necessary for normal nigrostriatal function. The demonstration that transplanted fetal monkey SN can ameliorate the MPTP-induced parkinsonism in monkeys (16) sets the stage for considering such an approach in humans with parkinsonism. However, the etiologic process responsible for the destruction of dopamine cells in parkinsonism may still be active and may destroy freshly implanted cells. Moreover, if the normal postsynaptic targets of dopamine have been destroyed then even viable dopamine-producing cells may not have any effect.

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EFFECT OF EGF ON DNA RNA SYNTHESIS AND PROTEIN ADP-RIBOSYLATION IN FETAL RAT BRAIN TISSUE SLICES
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Response of Amputated Rat Limbs to Fetal Nerve Tissue Implants and Direct Current

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Summary: We have previously shown that implanted fetal nerve tissue stimulates the regeneration of amputated chick limbs. The purpose of this study was to determine whether a similar phenomenon would occur in amputated rat limbs and if addition of applied direct current (DC) would affect this process. Thus, fetal nerve tissue was implanted into amputated stumps of 3-week-old rats; variable tissue regeneration was induced that was dependent on the age of the donor implant and the presence of applied DC. Twelve or 14 day fetal neural implants induced new accessory bones containing epiphyseal plates and marrow cavities and occasionally formed joint-like structures with the host humerus. Addition of DC to 12 day neural implants increased the number of new bones formed. Eighteen day neural tissue with applied DC did not induce new bone formation but stimulated the maximal elongation of the host humerus and outgrowth of nerve fibers to the cut surface. Implantation of fetal heart tissue or implantation of fetal neural tissue into unamputated limbs failed to induce new bone formation. Although true limb regeneration was not achieved, formation of new skeletal elements did occur and this effect was enhanced by applied DC. **Key Words:** Regeneration—Fetal implants—Rat limbs—Direct current.

Many attempts have been made to induce limb regeneration in higher vertebrates (adult frogs, chick embryos, newborn opossums, young rats). Experimental procedures that have been used in this pursuit include diversion of nerve fibers to the amputated limb (1,17), transplantation of adrenals (16), skin removal with application of salt solution (14), electrical stimulation (2,4,11,19,20), electrical stimulation with nerve growth factor injection (19), and implantation of fetal nervous tissue into the amputated stump (8,12). The only studies that demonstrated significant numbers of successful limb regenerates (including digits) were those of Mizell (12), who implanted fetal brain tissue prior to amputating the hindlimbs of newborn opossums, and our own studies of implantation of embryonic neural tube into amputated stumps of chick embryos (8). Additionally, reports of regrown fingertips of young children after amputation of digits distal to the last joint when the cut end was not sutured are significant (10). All of these studies indicate that higher vertebrates, including man, can exhibit varying degrees of regenerative ability under the appropriate stimulus (9).

Previous studies in rats by Becker (2), Sisken et al. (19), and Libbin et al. (11) demonstrated the extraordinary growth of the amputated humerus and tissue regeneration with formation of new skeletal elements after treatment with applied direct current (DC). The appearance of such new skeletal elements was also noted after the addition of embryonic nerve tissue in our chick embryo studies in

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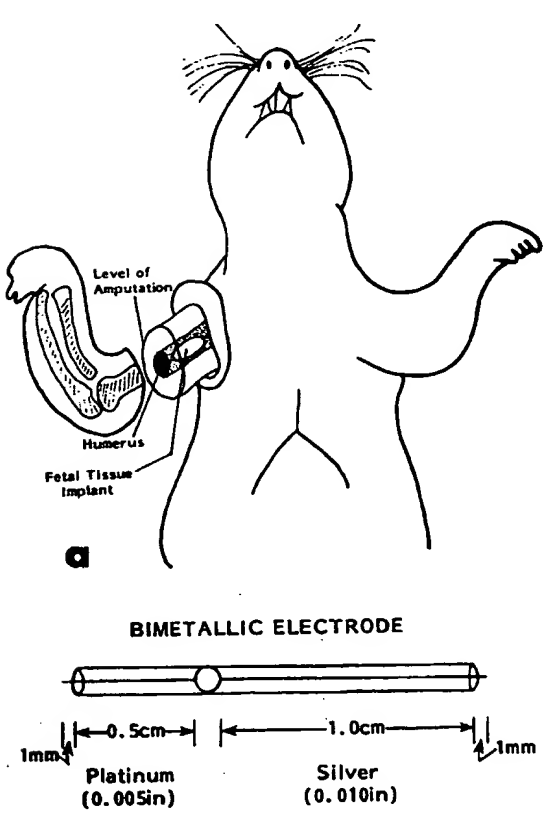

FIG. 1. Diagrams. a: Midhumeral amputation of a 3-week-old rat limb and implantation of fetal tissue next to the proximal portion of the humerus. b: Specifications of the bimetallic electrode (BME) insulated up to the final 1 mm on each end. In some cases both wires were 2× the lengths shown here. c: Relationships of implanted fetal tissue and BME to the anatomical structures of the limb.

those animals demonstrating partial, as well as complete, regeneration.

The aim of the present study was to use the same technique developed in the chick model (the insertion of fetal nervous tissue into the amputated stump) on the amputated rat limb. Not only did we test the inductive properties of implanted fetal nervous tissue alone but we also combined the implantation technique with the application of DC-yielding devices. We conclude from these studies that implanted neural tissue either alone or in combination with applied DC produces varying degees of tissue regeneration that appears to be correlated with the age of the fetal implant as well as the treatment imposed.

MATERIALS AND METHODS

To minimize rejection of implanted tissue, a highly inbred strain of rats (Harlan Fisher F_{344}; Harlan Sprague Dawley, Inc., Indianapolis, IN) was used. Limb amputations were performed on 3-week-old male rats, weighing approximately 50 g each. Donor tissue was obtained from fetuses of the same inbred strain.

The animals were anesthetized with 0.05 ml sodium pentobarbital (65 mg/ml, Butler Co., Columbus, OH) and the right forelimb shaved. The skin was incised circumferentially, then retracted proximally. After ligation of the brachial artery, the forelimb was amputated at the midhumeral level by means of blunt scissors (Fig. 1a). To facilitate future identification of the level of amputation, the distal segments were numbered and placed in neutral formalin.

Pregnant females of the same inbred strain containing fetuses of 12–18 days gestation were anesthetized and their abdomens swabbed with 70% alcohol. Through a vertical incision, the two uteri containing the embryos were removed and placed in sterile buffered saline solution. Each embryo was freed from its extraembryonic membranes and placed in sterile Minimum Essential Medium buffered with 25 m*M* HEPES (Gibco, Grand Island, NY).

The heart or central nervous system (CNS) was dissected from these embryos and placed in separate sterile dishes containing tissue culture Minimum Essential Medium. The neural tissue implant included the brain, spinal cord with primordia of the peripheral nervous system, or the neural crest, which differentiated into unipolar sensory ganglion cells. The somatic efferent neurons of the ventral gray of implanted spinal cord sent axons into the tissues of the host limb.

Bimetallic electrodes (BME) were used to deliver DC (20). These consisted of platinum wire (0.005 inch) fused end-to-end with silver wire (0.010 inch, Medwire Corp., Mt. Vernon, NY) and insulated with plastic that was removed from the ends of the electrodes before use (Fig. 1b). The BME were implanted into the stump by inserting the platinum end next to the cut humerus; the silver wire was fixed in the triceps muscle (Fig. 1c). *In vitro* measurements of the current/voltage characteristics of these BME were made with a Keithley Electrometer (Model 602) in the following manner: 2 cm lengths of platinum wire and silver wire insulated except at the tips were separated by a distance of 0.5 or 1 cm and suspended from the top of a 50 mm Petri dish into 3 ml sterile Minimum Essential Medium. The dish was placed in a 37°C incubator, and readings of the current generated by the two dissimilar metals were taken at 1–5 min intervals for the first 60 min, and hourly thereafter for the next 4 h. Constant current levels of 28–30 nA were obtained. Since the two electrodes were placed in the stump approximately 0.5 cm apart, the current density delivered by the BME was approximately 6 nA/mm^2.

Sixteen control animals were divided into three groups: six animals of Group I underwent limb amputation alone; five animals of Group II underwent limb amputation with fetal heart tissue implanted into residual stumps; five animals of Group III received fetal neural tissue implants but had no amputation.

Fifty-three experimental animals underwent amputation and were divided into four groups. Two (Group IV) were implanted with BME alone. Neural tissue from 12, 14, and 18 day fetuses was implanted into the limbs of 26 animals (Group V). This tissue consisted of cerebrum, brainstem, or spinal cord. Fifteen animals of Group VI received fetal neural tissue with BME (Fig. 1c). Ten animals (Group VII) had fetal nervous tissue and plain silver wire as a control for the BME. In both experimental and control animals, with the exception of Groups III and IV, the fetal implant was placed next to the ligated brachial artery and median nerve beneath the remnants of the biceps muscle (Fig. 1a). In Group III animals the implant was placed between the intact biceps and the subjacent artery and nerve. After amputation and implantation, redundant skin was sutured over the stump. The animals were individually housed in wire-bottom cages for periods of 1, 2, and 3 months. At the end of each time period, animals from each group were anesthetized, and the right limbs removed and fixed in 10% neutral formalin. Each limb was X-rayed following fixation. The limbs were then decalcified, dehydrated, and embedded in paraffin. Eight micron serial sections of each limb were cut, mounted on slides, and stained in hematoxylin and eosin. All sections were evaluated with respect to the extent of growth of the humerus beyond the original amputation site, the number of new bones appearing in the limb stump, the presence of histologically normal implant tissue, and the growth of peripheral nerve beyond the level of amputation. The total humeral length and the length of growth of the humerus beyond the amputation line were measured (in millimeters) on histological sections under a dissecting microscope. A Student's *t* test was used to determine significance of effect.

RESULTS

Control Animals

The controls consisted in part of animals in which amputated limbs received no treatment (Group I) and those that received an implant of 14 day fetal heart tissue (Group II). In addition, the inductive capacities of implanted fetal neural tissue were tested by implanting 14 and 18 day fetal neural tissue into intact limbs (Group III). In Group III fetal implants were found in eight of 10 specimens. In no case did new bones develop, although a small mass of cartilage, oval in shape, was found in one of the specimens. X-ray films of control animals without implants (Group I) or with implants of heart tissue (Group II) showed no new bones formed and limited growth of the severed end of the humerus (Fig. 2a–c). Histological examination of a typical control animal that received an implant of fetal heart tissue revealed healthy cardiac muscle located near and growing into the triceps brachii muscle of the amputated limb (Fig. 2d). The distal end of the humerus of this specimen demonstrated wound re-

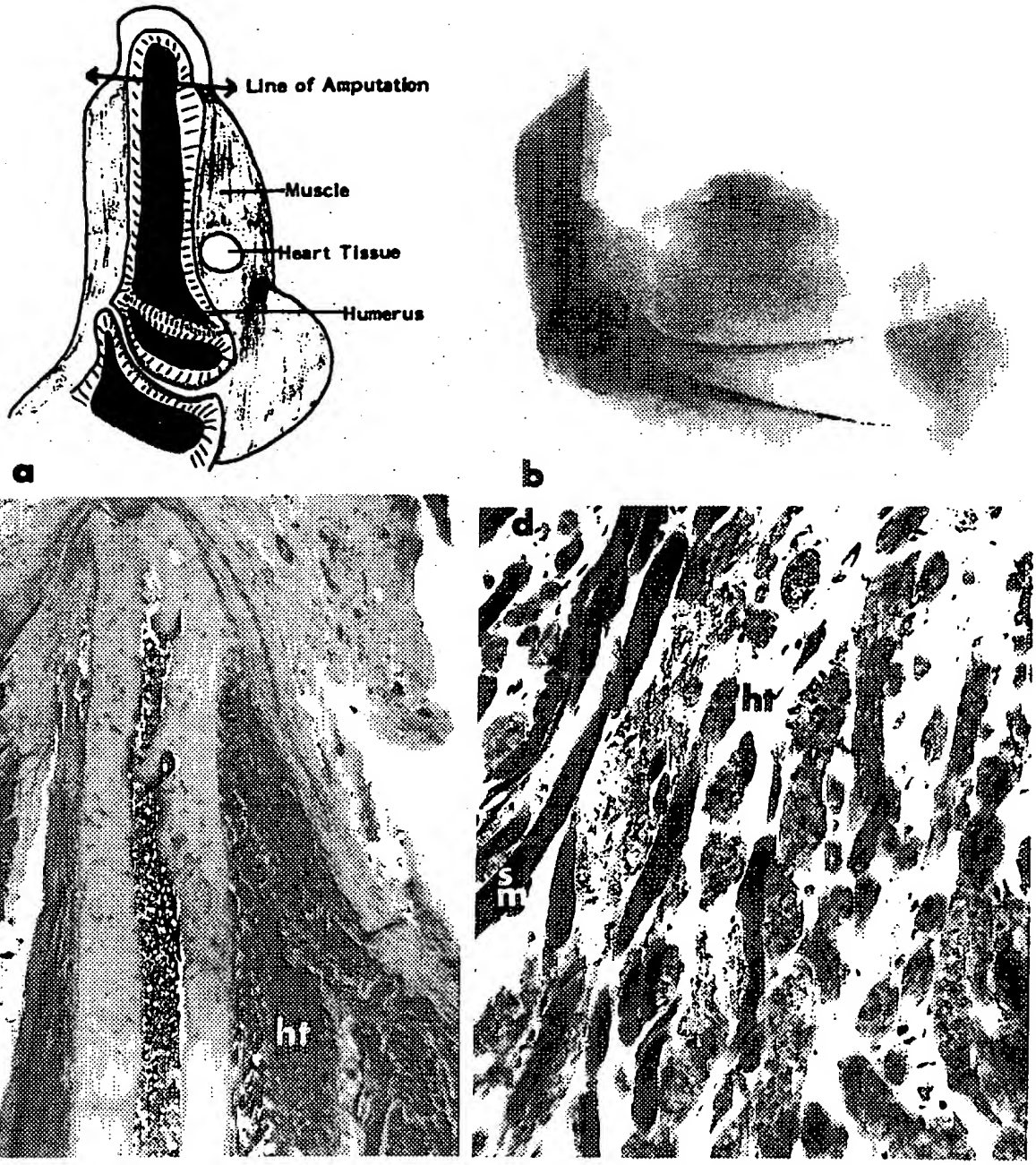

FIG. 2. Control rat, Group II, 2 months postamputation and implantation of 12 day fetal heart tissue. **a:** Diagram of the rat limb illustrates the line of amputation, location of the implanted heart tissue, and the healing of the cut surface of the amputated humerus. **b:** Photograph of X-ray film taken after fixation of the limb. **c:** Low-power photomicrograph of the histological section of this limb illustrates connective tissue growth over the cut surface of the humerus and the heart tissue (ht) growing between the humerus and the large skeletal muscle mass of the triceps brachii. ×14. **d:** Photomicrograph of the heart tissue (ht) growing next to and within the triceps skeletal muscle (sm). ×425.

TABLE 1. *Comparison of control and experimental animals*

Group	Implant	No.	Implant found	With new bones	Average No. bones
Control					
I	None	6	0	0	0
II	Heart	5	5	0	0
III	CNS without amputation	5	3	1[a]	0
Subtotal		16	8	1	0
Experimental					
IV	BME	2	0	0	0
V	CNS	26	22	18	3
VI	CNS + BME	15	10	3	7
VII	CNS + WIRE	10	7	4	2
Subtotal		53	39	26	0
Total		69	47	26	0

BME, Pt/Ag insulated bimetallic electrodes; CNS, fetal neural implant; WIRE, 0.005 inch insulated Pt or 0.01 inch Ag wire.
[a] Small amount of cartilage.

pair with dense irregular connective tissue adherant to surrounding large muscle masses (Fig. 2c). Figure 2a is a diagram that shows the position of the heart implant relative to host limb structures, the level of amputation, and the extent of humeral growth.

Experimental Animals

The experimental groups consisted of animals in which amputated limb stumps were treated to test the potential for elongation of the cut humerus and the induction of new bones (see Table 1 and Fig. 3). Two animals received a BME alone (Group IV). This group was made small since ample evidence from our previous work (19) had demonstrated that BME exposure did not stimulate new extra bones. The two animals used in this study conformed to the previous results. Twenty-six animals (Group V) had fetal neural tissue alone implanted in the stumps of the amputated limbs. This implant survived in 22 animals; the formation of new bones was noted in 18 of these (81.8%) with an average of three new bones in each limb. X-ray films of these animals showed the new bones near the distal end of the stump (Figs. 4a and b). On histological examination the bones were found on the anterior and posterior aspects of the humerus. In each case the new bones were juxtaposed to the fetal neural implant. The size and shape of the bones varied; all contained bone marrow and most had epiphyseal plates (Figs. 4c and d). Bones located near the distal end of the humerus frequently formed a joint like relationship between the new bone and the humerus (Figs. 4c and d and diagrammed in 4a). In specimens sacrificed 6 months after amputation, host skeletal muscle was attached to some of the ectopic bones so that movement of the new bones was possible.

Group VI consisted of 15 animals that received a fetal neural tissue implant together with a BME designed to deliver DC to the limb (Fig. 1c). The neural tissue implant and the electrode were found in 10 animals and new bones developed in three (30%) with an average of seven new bones in each specimen. The three animals in which new bones formed received an implant of 12 day fetal neural tissue whereas the seven animals in which new bones failed to develop each received an implant of 18 day fetal neural tissue. The combination of 12 day neural tissue and BME thus appears to produce the greatest response (Figs. 5a–d). The average number of new bones in these animals was larger than in those that received the CNS implant alone (Table 2). The fetal neural tissue appeared healthy when examined and was surrounded by the individual new bones many of which contained epiphyseal plates (Fig. 5d). The implant contained many large neurons and glial elements (Fig. 5e).

Although 18 day fetal neural implants in combination with BME failed to induce new bones (Figs. 6a and c), additional bone formed on the distal end of the humerus in such animals significantly exceeded that seen in control animals (Figs. 6b and d and Fig. 3). The absolute (length of growth in millimeters) and relative changes (percent of new

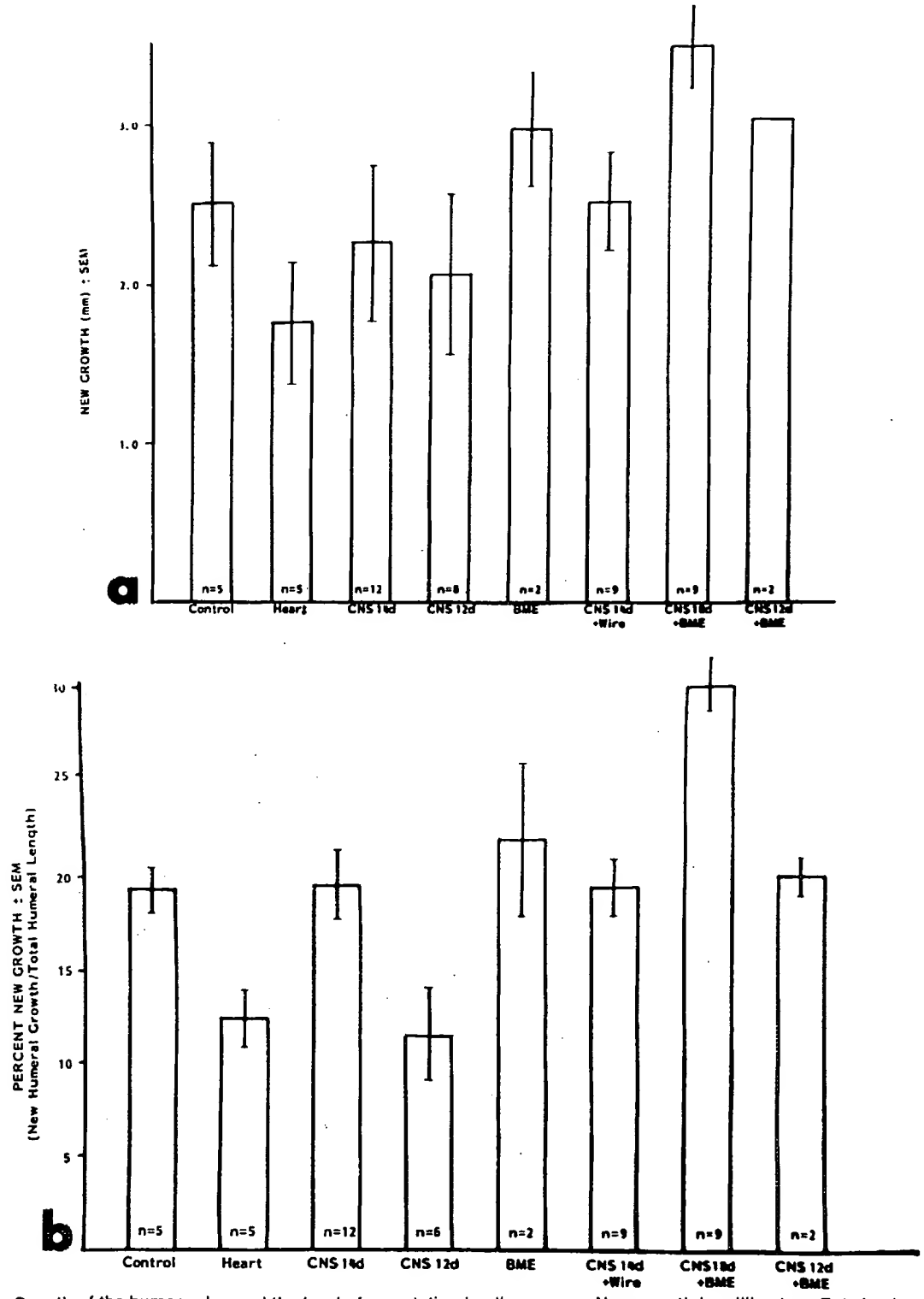

FIG. 3. Growth of the humerus beyond the level of amputation in all groups. a: New growth in millimeters. Rats implanted with 18 day fetal neural tissue with bimetallic electrode (BME) show a significant increase ($p = 0.05$) in length relative to the control (no implant) group. b: Percent new growth in millimeters (new growth/total humeral length). Rats implanted with 18 day fetal neural tissue with BME show a significant increase ($p = 0.025$) in growth relative to control animals (no implant).

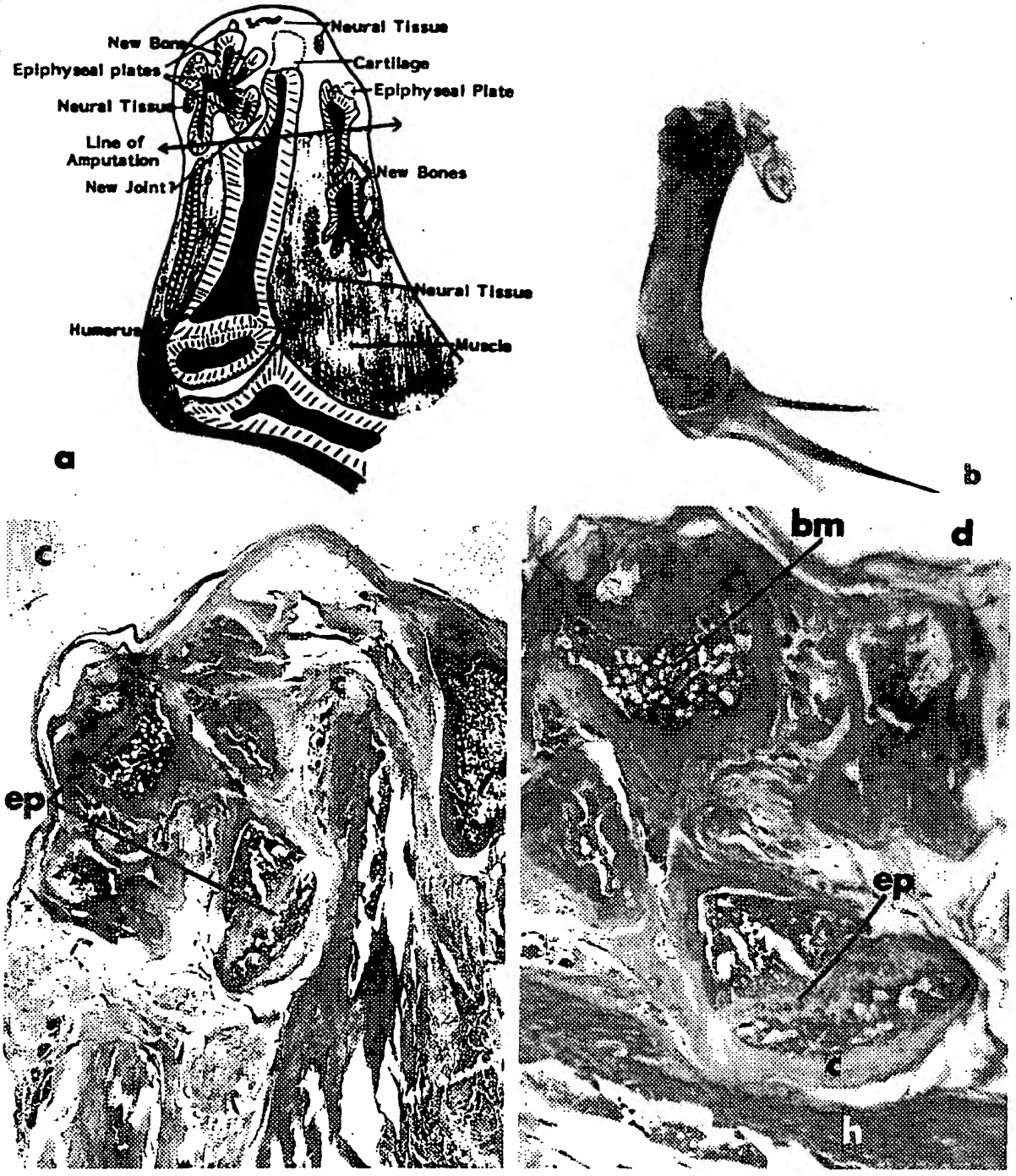

FIG. 4. Experimental rat, Group V, 2 months postamputation and implantation of 14 day fetal spinal cord. **a:** Diagram of rat limb illustrates the position of the new accessory bones relative to the implanted neural tissue and the distal part of the amputated humerus. **b:** Photograph of X-ray film of this specimen. Note the X-ray positive bones located on the anterior and posterior borders of the humerus. **c:** Low-power photomicrograph of a histological section of this limb. The new bones located near the anterior surface of the humerus contain epiphyseal plates (ep) and bone marrow; one new bone has formed a pseudojoint with the humerus. ×15. **d:** Enlargement of a portion of (c). Two bones contain cartilage (c), bone marrow (bm), and epiphyseal plates (ep). The joint-like relationship of one bone next to the humerus (h) is illustrated. ×30.

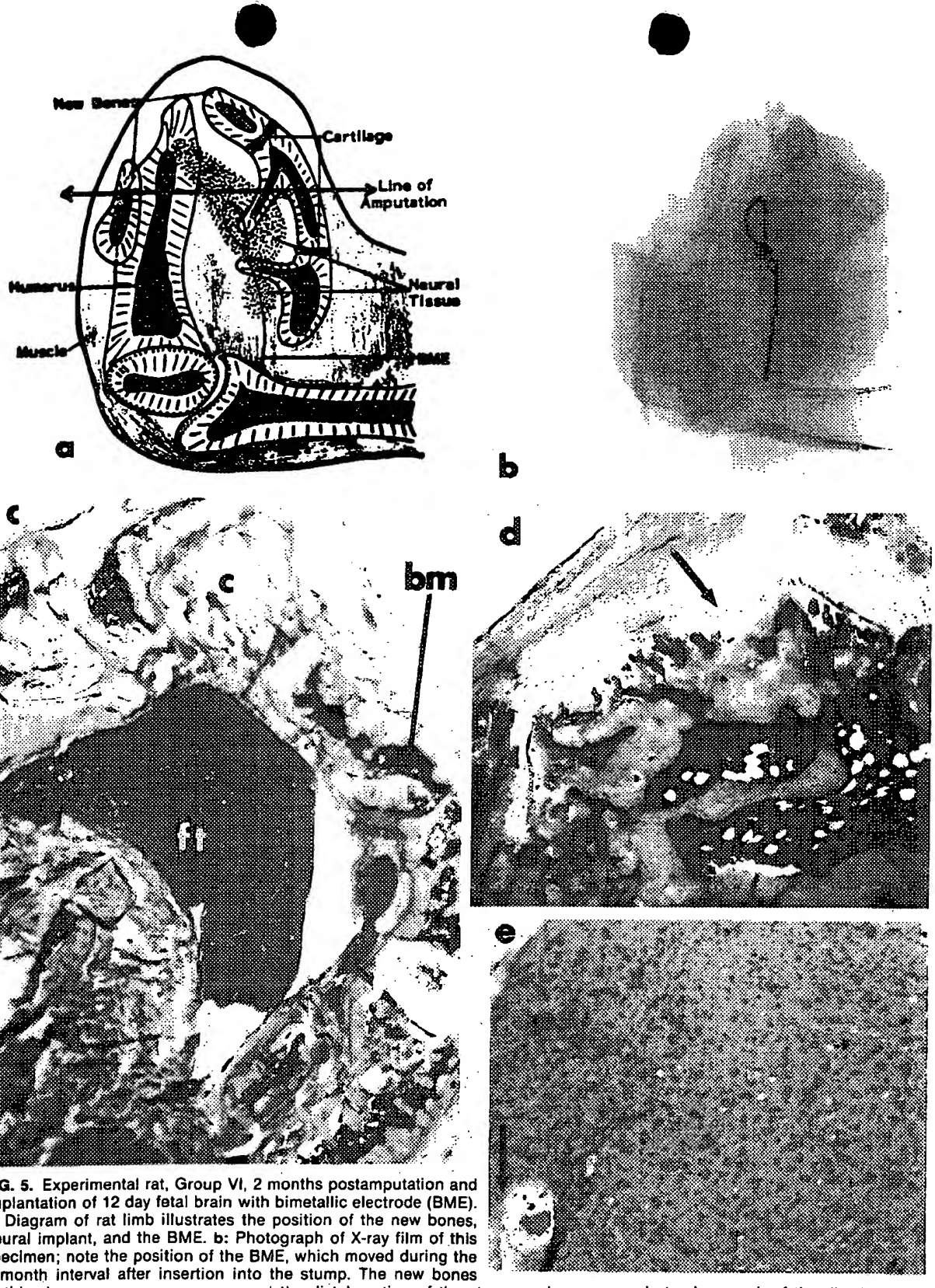

FIG. 5. Experimental rat, Group VI, 2 months postamputation and implantation of 12 day fetal brain with bimetallic electrode (BME). **a:** Diagram of rat limb illustrates the position of the new bones, neural implant, and the BME. **b:** Photograph of X-ray film of this specimen; note the position of the BME, which moved during the 2 month interval after insertion into the stump. The new bones in this view appear as gray masses at the distal portion of the stump. **c:** Low-power photomicrograph of the distal portion of the stump that contains new bones with bone marrow (bm) and cartilage (c) juxtaposed to, and formed around, the fetal neural implant (ft). ×32. **d:** Deeper section of new bone in (c) demonstrates a wide epiphyseal plate (arrow). ×80. **e:** Enlargement of the fetal implant in (c) to show the presence of large numbers of neurons. A portion of the implant (arrow) has grown into the underlying tissue, whereas the rest of the border is distinct. ×192.

TABLE 2. *Effect of age of fetal implant on formation of new bones*

Fetal implant age (day)	No. implanted	Implant (group)	Implant found	With new bones	Average No. new bones
12	9	CNS (V)	9	9	4
	3	CNS + BME (VI)	3	3	7
	2	Heart (II)	2	0	0
Subtotal	14		14	12	4.75
14	17	CNS (V)	13	9	2
	9	CNS + WIRE (VII)	7	4	2
	3	CNS without amputation (III)	3	1[a]	0
Subtotal	29		23	13	2
18	12	CNS + BME (VI)	7	0	0
	1	CNS + WIRE (VII)	0	0	0
	2	CNS without amputation (III)	0	0	0
	3	Heart (II)	3	0	0
Subtotal	18		10	0	0
Total	61		47	25	3.32

See Table 1 for explanation of implant.
[a] Small amount of cartilage.

length/total humeral length) in the various groups sacrificed at 2 or 3 months postamputation were measured on histological sections and are presented in Fig. 3. In all cases maximum growth of bone at the severed end of the humerus occurred in the rats implanted with 18 day fetal neural tissue along with the BME. In addition, the BME appeared to enhance the growth of nerve fibers into the distal portion of the limb (Fig. 6d), a phenomenon previously noted when BME was implanted in amputated rat limbs (19) and when agar salt-bridge electrodes were implanted into amputated frog limbs (4).

Group VII consisted of 10 animals that received fetal neural tissue plus a single wire to investigate the effects of mechanical irritation on the bone-inducing capacity of fetal neural tissue. The implant was found in seven animals, and four of these (57%) contained new bones with an average of two bones each. Comparing these results with those of Group V, the single wire did appear to depress the formation of new bones by the fetal implants, while not affecting humeral growth to any extent.

There were obvious differences in the bone-inducing capacities of fetal neural tissue at the development ages used in this study (see Table 2). The graft survival was best and formation of new bones was greatest with 12 day fetal neural tissue. When CNS alone was implanted, all nine implants lived and all nine contained new bones with an average of four new bones in each limb. In combination with BME three of three 12 day implants survived with an average of seven new bones in each limb. Using neural implants from 14 day fetuses, the survival rate dropped from 100 to 69% (nine of 13) with CNS alone and to 57% (four of seven) with CNS combined with BME with the average number of new bones reduced to two. With 18 day fetal neural tissue in combination with BME, there was a total failure to induce formation of new bones. However, new growth of the cut humerus was stimulated to the greatest degree in these animals. A synergistic action occurs with implanted BME and 18 day neural tissue since BME alone was not as effective as the combination of BME and older neural tissue. Thus, it appears that maximal tissue regeneration occurs with the younger neural implants in combination with BME, whereas maximal humeral growth is achieved with the older neural implants in combination with the BME.

DISCUSSION

We have found that implantation of 12–14 day fetal neural tissues into amputated stumps of young rats induced the formation of multiple new bones containing bone marrow and epiphyseal plates. In each case the sites of these new skeletal elements correlate with the presence of differentiating fetal nervous tissue. In contrast, implants of fetal heart tissue induced no new bone formation. In animals implanted with neural tissue some of the new bones were juxtaposed to the severed end of the host hu-

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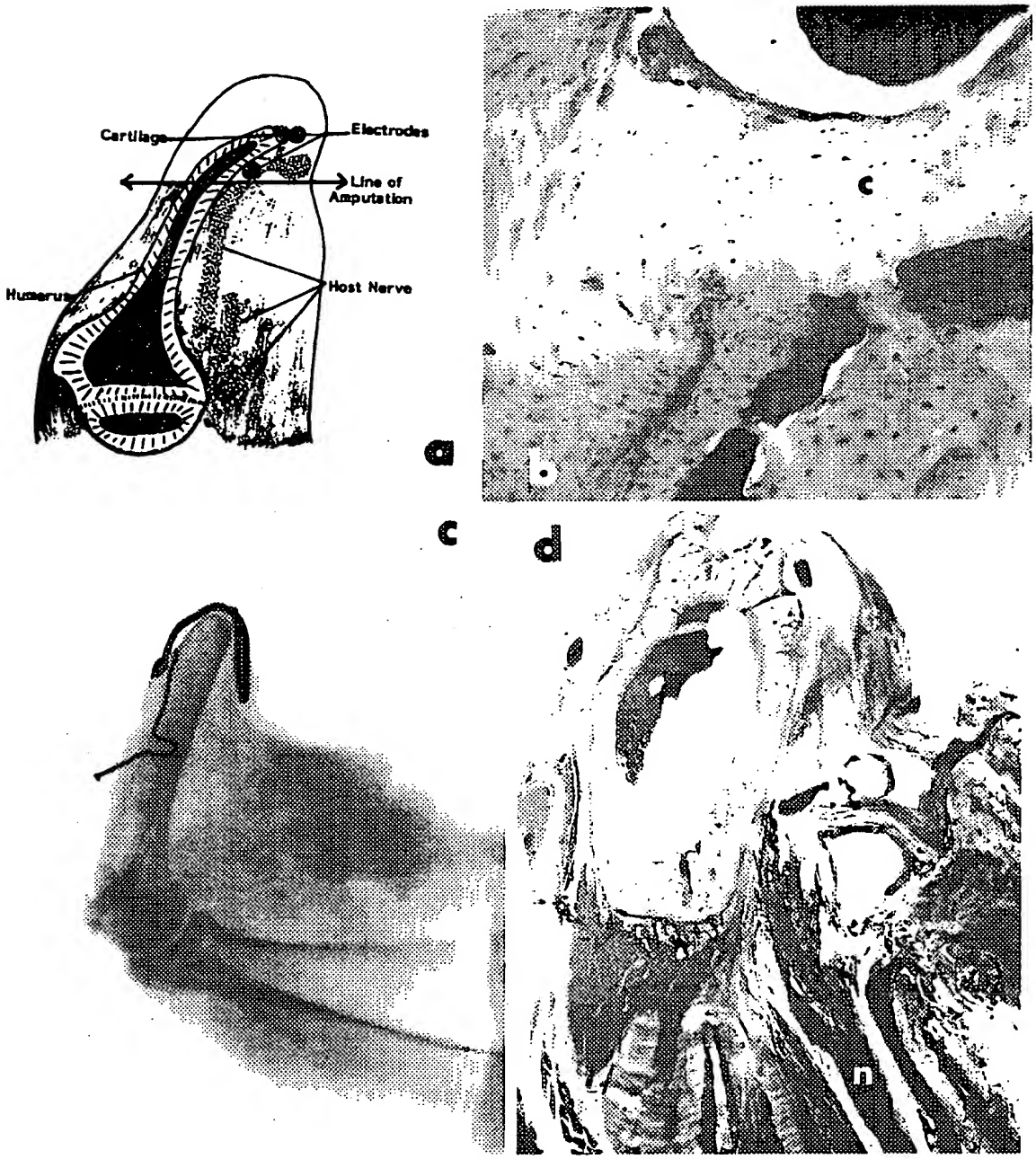
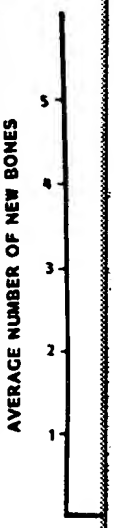


FIG. 6. Experimental rat, Group VI, 2 months postamputation and implantation of 18 day fetal spinal cord with bimetallic electrode (BME) **a:** Diagram of rat limb illustrates cross sections of the BME, elongation of the humerus beyond the line of amputation, cartilage at the end of this growth, and excessive host nerve tissue growing out toward the regenerated area. **b:** Enlargement of the most distal portion of the growing humerus. Cartilage (c) next to the electrode has been invaded by blood vessels. ×374. **c:** Photograph of X-ray film of this specimen shows the final position of the BME at the time of sacrifice. **d:** Low-power photomicrograph of the most distal portion of the stump that includes the growing end of the humerus. The cross-sectioned electrodes are found next to the new bony portion of the humerus. Bundles of host nerve fibers (n) have grown into this area. ×28.

merus, so that a pseudojoint was formed. The final location of the fetal CNS could not be controlled, perhaps due to limb movement. Therefore, structures resembling joints were found only in those animals in which the implant grew near the distal end of the humerus.

The average number of new bones correlates directly with the age of the fetal nerve implant. The



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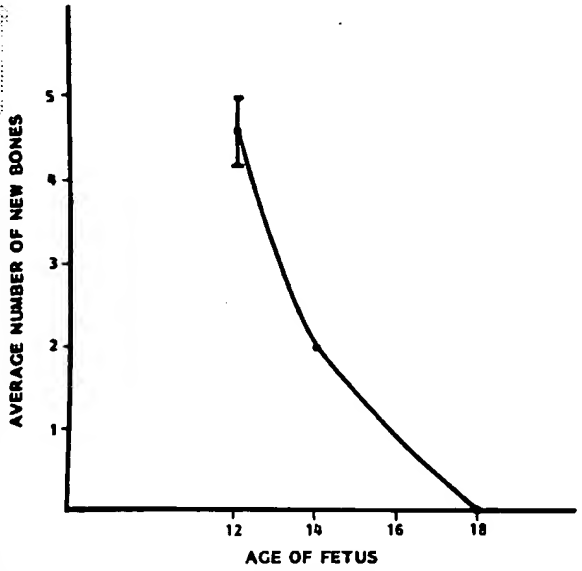

FIG. 7. Correlation of the numbers of new bones induced with the age of the fetus from which the implant was taken. The highest number of new bones formed (4.75) is obtained with 12 day fetal neural tissue implants.

younger the fetus from which the implant is taken, the greater the number of new bones formed (Fig. 7 and Table 2). These results suggest that younger neural tissue implants either grow better after transplantation than do older implants or have a greater capacity to induce new bones. Our material suggests that both factors may be involved. The younger implants contained fewer necrotic areas and appeared to grow larger than the older implants, demonstrating improved survival and proliferation. New bones found in association with the younger implants showed a greater tendency to develop in direct contact with the neural tissue than did those bones formed in response to the older implants. The effects noted may be interpreted as an indication of reduced inductive capacity as the neural tissue ages. Results of experiments in the chick embryo (unpublished results) show that by 8 days of incubation the neural tissue no longer has the capacity to induce regeneration of the middle and distal limb segments. This is in contrast to demonstrated inductive capacities at 2 and 4 days.

In Table 2 data obtained from animals with implanted fetal neural tissue alone were compared with that obtained from animals implanted with fetal neural tissue and BME. The combination of 12 day nerve implants with BME yielded the greatest number of new bones, seven, formed per animal. The source of the cells forming these new bones has not been ascertained. Mesenchymal-like elements are likely candidates. These results are consistent with the well-documented effects of electric current on bone repair and new bone formation in animal models and in humans (reviewed in 22).

The results obtained in this study are not as dramatic as those obtained in chick embryos in which implantation of 2 day embryonic neural tube into 4 day amputated limbs (Type III response) resulted in regeneration of middle and distal segments. The growth of several tissues, however, is significant as it demonstrates that the rat has the potential to respond to provocative signals by forming completely new tissue. The response that we obtained in the young rat is directly comparable with the "Type II response" that was seen in chick embryos (8). In this group the implantation of 2 day neural tube resulted in tissue regeneration only, producing extra bones in the proximal segment.

In 1968 Mizell (12) reported that implants of nervous tissue effectively induced regeneration of the distal segment of hindlimbs of the newborn opossum. At birth the hindlimbs of the opossum are essentially in the fetal stage, so that one can assume that mammalian fetuses are able to undergo epimorphic regeneration under unique experimental conditions. Our objective was to determine the extent to which an older mammal can respond to implanted neural tissue.

This investigation is not the first to show that the rat is capable of extraordinary repair and has the capacity to form new bones. In 1972 Becker (2) demonstrated ectopic bone formation in amputated rat forelimbs as a result of DC stimulation. These bones were formed rapidly and disappeared with age. However, new epiphyseal plates at the cut end of the humerus were prominent. In 1979 Sisken et al. (19) and Libbin et al. (11) reported similar results using DC provided by BME in amputated rat forelimbs. Both groups of investigators found extraordinary epiphyseal plate formation on the distal portion of the humerus.

In our 1979 study we also injected nerve growth factor (NGF) into the amputated limbs of a group of animals; in 40% of the animals ectopic bones were formed. These new bones were small and were found only in rats sacrificed 1 month after amputation. The induction of new bones after administration of a neurotropic substance such as NGF or implantation of neural tissue supports the thesis proposed by Singer (18). He suggested that nerve tissue in the limb secretes neurotrophic substances that may be involved in the stimulation of

tissue and limb regeneration. The greater the number or caliber of nerves per unit amputation area, the larger the amount of trophic agent delivered. He and Rzehek found, for instance (15), that in the mammal (mouse) the limb contains only 16% of the number of nerves per unit area of amputated limb as that found in the newt. These nerves are also thinner. Such a paucity of innervation to an amputated limb would probably result in insufficient neurotrophic substance delivered, and thus a lack of regenerative ability. Augmentation of the innervation of the limb by implanted neural tissue appears to be a logical method to increase this ratio and in newborn opossum and chick embryos appears an adequate substitute for the nerve fiber deficiency, thereby facilitating replacement of the distal limb segments. However, in our experiments using older mammals there is an indication that an increase in nerve supply alone is inadequate to induce complete limb regeneration. New skeletal elements were found that could perhaps represent future elements of middle and distal segments, but true limb regeneration did not occur.

It is not surprising that new skeletal tissue was found after DC administration. Many cases have been cited in the literature on electric field stimulation of cartilage and bone *in vivo* and *in vitro* (2–4,6,7,11,13,19–22). In addition, the importance of the nervous system in bone growth has been illustrated by the experiments of Bunch et al. (5). Denervation of rat hindlimbs followed by below-knee amputation reduced the mass and length of the amputated tibia. There was a direct correlation between the absence of innervation and the decrease in rate of periosteal mitosis. In the present study supplementing the neural tissue implant with DC stimulated new mesenchymal differentiation and neurite outgrowth from the host peripheral nerves. However, this combination of treatments stimulated greater numbers of ectopic bones but failed to induce the proper relations of new skeletal elements in the amputated limbs.

The mechanisms by which implanted fetal nerve tissue stimulates osteogenesis in amputated limbs are as yet unknown. It appears from our work that amputation is necessary to activate this process, for implantation of fetal nerve tissue in normal limbs failed to induce ectopic bone formation. Our hypothesis is that bone formation may be induced by trophic substances produced by the implanted nerve cells. These substances may be similar to those that induce complete regeneration of amputated limbs in other vertebrates. Our observations indicate a direct relationship between the age of the nerve implant and the number of new bones formed, and an inverse relationship between the age of the implant and the number of neurons that survive. That is, the younger the implant, the more surviving neurons available to secrete trophic factors that induce a greater number of new bones.

This approach to the study of mammalian limb regeneration does appear to offer some hope for future success based on the following observations: (a) Many of the ectopic bones formed had the structure of a long bone, that is, they had an epiphyseal plate at each end, forming two epiphyses separated by a diaphysis. (b) In some cases there were apparent attempts to form synovial joints between the ectopic bones or between these bones and the host humerus. (c) Several of the specimens showed that host skeletal muscles achieved an attachment to the ectopic bones so that movement of the new bones could occur.

Functional replacement of a whole limb after ablation, which occurs naturally in salamanders, has yet to be accomplished in adult mammals. Future studies to determine the basis of the inductive capabilities of implanted fetal nerve tissue and applied DC on the reactive stump tissue should provide information to assist in achieving the ultimate goal of limb regeneration in adult mammals.

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09277876 PASCAL No.: 91-0068251
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1990

45/6/2 (Item 2 from file: 155)
06469018 90157786 PMID: 1968181 Record Identifier: 30254
Judicial warning on very late abortions .
Feb 24 1990

45/6/3 (Item 3 from file: 73)
04079873 EMBASE No: 1989248919
Voluntarily inflicted penetrating uterine wounds in pregnancy: A case report and review of the literature
1989

45/6/4 (Item 4 from file: 155)
05702018 88120918 PMID: 2893230 Record Identifier: 27571
Fetal spare parts.
Feb 20 1988

45/6/5 (Item 5 from file: 155)
05363251 87116825 PMID: 3808533
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Mar 1987

45/6/6 (Item 6 from file: 155)
04399098 84085760 PMID: 6557981
[Cadmium and lead concentrations in the amniotic fluid of pregnant smokers and non-smokers]
Cadmium- und Bleikonzen-trationen im Fruchtwasser von rauchenden und nicht-rauchenden Gravida.
Nov 1983

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04327047 84010762 PMID: 6225871 Record Identifier: 16190
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Sep 1983

45/6/8 (Item 8 from file: 5)
03823560 BIOSIS NO.: 000075001633
IMMUNO REACTIVE BETA ENDORPHIN LIPOTROPIN IN THE CHRONICALLY CANNULATED OVINE FETUS RESPONSE TO BILATERAL FETAL ADRENALECTOMY
1982

45/6/9 (Item 9 from file: 5)
03236992 BIOSIS NO.: 000071050103
A CASE OF PURE RED CELL APLASIA ASSOCIATED WITH PREGNANCY AND BONE MARROW CELLS RESPONSIVE TO ERYTHROPOIETIN IN-VITRO
1980

45/6/10 (Item 10 from file: 155)
02803129 78121357 PMID: 629293
Human fetal respiration. IV. Failure of severe distress to stimulate aspiration of amniotic fluid by the immature human fetus.
Feb 15 1978

Medicine and the Law

Fetal Spare Parts

ATTENTION has lately focused on aborted healthy fetuses as potential sources of organs or tissue for transplantation in, for example, patients with Parkinson's disease. What legal issues might such a practice raise? English law recognises that in the fetus there is a potential human being developing, but until it has an existence separate from its mother it does not acquire full human status.[1]

The Abortion Act 1967 altered the law protecting the right of an immature fetus, which is not "viable", to develop in the womb. Mr David Alton's Private Member's Bill (given its second reading on Jan 22) seeks to reverse this trend, and to reduce the period for lawful abortion to 18 weeks. At present, viability is deemed *prima facie* to occur at 28 weeks (under the Infant Life Preservation Act 1929), but if a fetus of less than 28 weeks is shown to be viable termination of the pregnancy is not lawful. In practice abortion after 24 weeks is very unusual; Prof Glanville Williams (*Times*, Jan 20, 1988) notes that only 4 such terminations were done during the last quarter of 1987.

In the UK some 165 000 legal abortions are performed each year, mainly for "social" reasons. Few are done at more than 20 weeks. Fetal remains are usually destroyed though some are used for research. Abortion is perceived of as a negative and depressing event, and the chance to give it a positive aspect, by making use of fetal material for helping the sick, is to be welcomed. If we use donated organs and tissue from dead human beings, why not from aborted fetuses, who have not gained the full status of personhood? The anti-abortion lobby sees it differently. Those who regard human life as beginning at conception (a legal concept accepted by Irish law and constitution) view the donation of tissue or organs from aborted fetuses as condoning abortion and find it unacceptable precisely because it does introduce a positive factor that might provide a sop to the consciences of waverers.

Difficulties could arise if a woman were to choose to become pregnant with a view to having an abortion to help, say, a sick member of the family who might benefit from fetal tissue or organs. It might seem both unethical and undesirable for a woman to become pregnant for this purpose—it would be a manipulation of the process of creation of life, analogous to the rule that embryos should not be specifically created for research processes. A woman with this intention could pretend that the unwanted pregnancy was unplanned. She alone would bear the uneasy burden of the truth though her doctors might well be suspicious and reluctant to collaborate. But should such a woman be censored? She can decide to conceive with the object, not of having a child but of helping someone else, and so risk censure or refuse knowing that someone in her family has been allowed to deteriorate mentally or even die when he or she might have been saved. A feckless woman who became pregnant by casual intercourse could then ethically provide fetal tissue, whereas a caring woman who planned the event could not.

Using normal organs or tissues from an abnormal fetus found by prenatal diagnosis in an otherwise wanted pregnancy is different. As Harrison points out,[2] "Organ transplants could give an increasing number of children with fatal childhood diseases the chance of a full life". He goes on to discuss the anencephalic fetus as a source for organ transplants but points out that there are difficult ethical and legal problems. Organs must be removed before all bodily functions cease or they may become useless for transplantation. Continued ventilation after cessation of spontaneous breathing is a possible option. However, the medical and legal status of the anencephalic fetus, both in the womb and at birth, is central to the argument. The confusion is all too well illustrated by the 1987 case in the United States of a woman who was found to be carrying an anencephalic fetus. She altruistically decided to take the pregnancy to term so that her baby's organs could be used for transplantation, though her decision, sadly, was to be in vain.

There are some statutory and judicial indications as to what qualities are necessary for a baby to be "viable" and amount to a "reasonable creature", but the final decision must take account of medical evidence, the ethics of the day, and commonsense. An anencephalic baby which sustains respiration at birth may be regarded as born alive, born dying, or "born dead". But is it a "reasonable creature", the destruction of whom outside the womb might be seen as murder? In the 17th century Lord Coke defined murder as "Where a person of sound memory and discretion—unlawfully killeth—any reasonable creature . . .". The legal text "Smith and Hogan" states that "reasonable creature" includes any human being, while in "Archbold" (another well-known legal authority) "reasonable" is said to relate to appearance rather than the mental capacity and "is apt to exclude monstrous births". The abnormal appearance of anencephalic babies and their lack of brain suggest that the anencephalic is not "a reasonable creature" in being. In Butterworths' *Medical Dictionary* "anencephalus" is defined as "A monster without a brain".

Is an anencephalic baby "in being" (ie, alive)? The law accepts that death has occurred when a doctor declares that it has. The era of transplantation gave new impetus to the notion of brainstem that in some circumstances has superseded the traditional criteria of cessation of the heart beat. Anencephalics never had and can never possess will or willpower or senses. Many are stillborn but some survive for a short period which can range from minutes to days. None survive long-term and no effort is made to sustain them. However, there is brainstem function. Should this be interpreted as conclusive evidence of human life? In my view, not necessarily. Anencephalics lack from the start the areas of the brain which provide the capacity to reason or feel pain, for example, which are pre-supposed to exist or to have existed in the definition of brainstem death that was developed to apply to normally constituted human beings who are dying or already dead.[3]

When an anencephalic baby is born with signs of breathing and circulation which persist for several minutes it will usually be registered as a live birth, possibly more for social reasons rather than as a reflection of medical reality. Even so the practice is indicative of the uncertainty of those concerned. Arguably, anencephalic fetuses have no protection under the law because they are not able to develop into human beings capable of sustaining independent life, and some doctors might not regard a full-term respiring anencephalic as a live baby. It is nonetheless the product of a human union, has a human form, and instinctively one feels that it should be allowed to die undisturbed without its existence, such as it is, being cut short by organ and tissue removal. To deny it humanity on the ground that it lacks reason is to open the question of who else may be excluded by means of neurological deficit or mental capacity which could be harmful to society as a whole.

The law is uncertain. Any doctor who removes organs from a "live" anencephalic baby might be open to a charge of murder even though his destruction of a mature anencephalic fetus while in the womb or being born would probably not amount to child destruction. The doctor could argue that an anencephalic child while still in the womb is not expected to be born alive so there would be no intent to destroy a "viable" fetus. Once this child has gained a separate existence from its mother, however tenuous, it may have to be regarded as a dying person. Ventilation would then be necessary after respiration and circulation cease, if the organs were to be used. A working group chaired by Sir Raymond Hoffenberg has been looking at the problems arising from organ transplantation in the newborn, and a report from the Conference of Medical Royal Colleges and their Faculties in the UK has just been published by the Department of Health and Social Security.

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Transplantation of fetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's disease

NEJM vol 318 issue 1

Madrazo (Author)

D haplotype, respectively, will carry the cystic fibrosis mutation. A person with a BB genotype has a 1 in 5 chance of carrying the cystic fibrosis gene, whereas a person with a CC genotype has a 1 in 500 chance.

These data are quite useful for evaluating pregnancies involving close relatives, such as aunts, uncles, and siblings of patients with cystic fibrosis. Combining these linkage-disequilibrium data with conventional linkage analysis of the parent at high risk and with microvillar intestinal-enzyme analysis[3] will reveal data on risk. For example, if a sibling of a patient with cystic fibrosis is found to contribute the cystic fibrosis mutation to the fetus and if the microvillar intestinal-enzyme analysis is abnormal, we calculate the probability of cystic fibrosis in the fetus to be 0.84 if the spouse contributes the B haplotype but only 0.04 if the spouse contributes the C haplotype. These probabilities assume 8 percent and 2 percent false negative and false positive rates, respectively, for microvillar intestinal-enzyme analysis. Obviously, better diagnosis will be possible when the cystic fibrosis mutation can be detected directly, and close relatives of patients with cystic fibrosis may wish to delay reproduction briefly in the hope that such detection will soon be possible. Meanwhile, the analyses described above will be useful, and it is important that 97.5 percent of families are informative for DNA analysis with tightly linked probes.

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TRANSPLANTATION OF FETAL SUBSTANTIA NIGRA AND ADRENAL MEDULLA TO THE CAUDATE NUCLEUS IN TWO PATIENTS WITH PARKINSON'S DISEASE

To the Editor: During wide experience with autografting of the adrenal medulla to the caudate nucleus in patients with Parkinson's disease,[1,2] we observed a marked improvement in young patients but a high morbidity and mortality rate in elderly patients (>60 years). The latter observation is probably due in part to the fact that autotransplantation involves two major, simultaneous operations, a laparotomy and a craniotomy, which at times makes postoperative recovery difficult in older patients. Since Parkinson's disease occurs predominantly in older patients, transplanting fetal tissue could be an alternative, particularly because such transplantation has been quite successful in various animal models,[3] and would considerably reduce surgical risk. We present a preliminary report on the courses in two patients with Parkinson's disease who received transplants of human fetal tissue to the brain.

Approval was obtained from the ethics and research committees of our hospital, and written consent from the patients and their relatives. Both patients were hospitalized until organ donation was possible. In the meantime, they were evaluated by means of video, CT scanning, electromyography evoked potentials, neuropsychological testing, and the Unified Parkinsonism Rating Scale (UPRS). On September 12, 1987, a 31-year-old woman admitted to the obstetric clinic with a history of repeated abortions due to cervicouterine incompetence had a spontaneous abortion after 13 weeks of pregnancy. After fetal death was certified by two physicians who were not part of the neurosurgical team, written consent for cadaveric organ donation was obtained from the woman. The two patients with Parkinson's disease, who had been maintained on intravenous cyclosporine and steroids, were operated on simultaneously. One patient (Case 1) received the fetal substantia nigra, and the second patient (Case 2) received the fetal adrenal medulla. In both cases the grafted tissue was placed within a cavity of the right caudate nucleus but in contact with the cerebrospinal fluid, according to a technique previously described.[1]

Case 1 was a 50-year-old man in whom Parkinson's disease had evolved over nine years. He had a score of 59 points on the UPRS while being treated with 1000 mg of Sinemet (levodopa–carbidopa), with predominance of rigidity and tremor. Case 2 was a 35-year-old woman who had had Parkinson's disease for five years. She had a score of 71 points on the UPRS while under treatment with 750 mg of Sinemet, with predominance of rigidity and bradykinesia.

After surgery and to date, both patients have been maintained on oral cyclosporine (2 mg per kilogram of body weight per day) and prednisone (15 mg per day) on a daily basis. At eight weeks after surgery, neither patient has had any complications. Case 1 has improved to the point of having a score of 45 points on the UPRS, and Case 2 has a score of 35 points. There has been an evident objective improvement in the symptoms of Parkinson's disease in both cases.

If long-term follow-up of these patients demonstrates sustained clinical improvement without complications, the use of fetal tissue as donor grafts may prove superior to autografting to treat Parkinson's disease.

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CANCER SURVEILLANCE IN PATIENTS WITH ULCERATIVE COLITIS

To the Editor: The critical review on cancer surveillance in ulcerative colitis (June 25 issue)[1] is an excellent summary of current knowledge. However, patients and physicians are left with a problem: Should those at high risk for cancer be screened for dysplasia? Analyses of findings from patients enrolled in our surveillance program indicate that screening provides certain benefits that should be offered to high-risk patients.

The University of Chicago surveillance program has existed for 10 years and has enrolled 99 patients who had pancolitis for an average of 17 years at the time of entry. In addition to the extent and duration of the disease, being older at the onset of symptoms was found to increase the risk of cancer.[2] Furthermore, the hazard rate (a measure of risk) was quantitated. For a patient with pancolitis beginning at the age of 30, the cancer risk is 5.2 percent during the 30th year of disease.

Daniel Davis

ORGANISMS: Hominidae (Hominidae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): animals; chord
mammals; primates; vertebrates
MISCELLANEOUS TERMS: ELECTIVE **SUCTION ABORTION** ; IMM
DISSECTION ; STRIATAL **TRANSPLANT** ; VENTRAL MESENCEPHA
CONCEPT CODES:
10612 External Effects-Physical and Mechanical Effects
11105 Anatomy and Histology, General and Comparative-Su
11107 Anatomy and Histology, General and Comparative-Re
Transplantation (1971-)
12512 Pathology, General and Miscellaneous-Therapy (197
13012 Metabolism-Proteins, Peptides and Amino Acids
17020 Endocrine System-Neuroendocrinology (1972-)
20504 Nervous System-Physiology and Biochemistry
20506 Nervous System-Pathology
25502 Developmental Biology-Embryology-General and Descriptive
25504 Developmental Biology-Embryology-Experimental
10060 Biochemical Studies-General
10064 Biochemical Studies-Proteins, Peptides and Amino Acids
16506 Reproductive System-Pathology
BIOSYSTEMATIC CODES:
86215 Hominidae
?t25/7/3,11,12,19,30,32,36,37

25/7/3 **(Item 3 from file: 5)**
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07250910 BIOSIS NO.: 000090030786
TRANSPLANTATION **OF HUMAN FETAL DOPAMINE CELLS FOR PARKINSON'S DISEASE RESULTS AT 1 YEAR**
AUTHOR: FREED C R; BREEZE R E; ROSENBERG N L; SCHNECK S A; WELLS T H; BARRETT J N; GRAFTON S T; HUANG S C; EIDELBERG D; ROTTENBERG D A
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ABSTRACT: In an effort to improve the clinical signs of Parkinson's disease, we have **implanted** mesencephalic dopamine cells from a 7-week human embryo into the caudate and putamen of a 52-year-old man with Parkinson's disease. **Fetal tissue** was obtained from elective **abortion** . The woman and the patient with Parkinson's disease were unknown to each other. The woman gave specific consent and was not paid. The patient had a 20-year history of parkinsonism treated with multiple drug therapies including levodopa/carbidopa (Sinemet) every 2 1/2 hours. His symptoms were worse on the left side. For 5 months prior to **transplantation** , the patient underwent clinical evaluations by both a neurologist and a computer system installed in his home for daily measurement of walking and hand movements. Preoperative positron emission tomographic scanning with 6-L[18F]fluorodopa (fluorodopa) demonstrated severe dopamine depletion bilaterally. **Fetal tissue** was matched to the patient for ABO blood antigens, and maternal serum was screened for hepatitis and human immunodeficiency virus type 1 prior to surgery. **Fetal tissue** was **implanted** stereotactically throughout the caudate and putamen on the right side of the brain via 10 needle tracks. The patient was not immunosuppressed. Results 12 months after surgery showed 42% improvement in left-hand speed before the first morning dose of drug and 40% greater response to drug therapy. Right-hand speed increased 15% before drug therapy and 23% after drug therapy. Reaction time was unaffected. Walking speed increased 33% after drug administration, although walking speed before the first morniing dose of drugs declined 40%. Walking speed on an all-day basis improved 17%. "On" time increased from 69% to 86% of the day. For technical reasons, preoperative and

Transplantation of Human Fetal Dopamine Cells for Parkinson's Disease

Results at 1 Year

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In an effort to improve the clinical signs of Parkinson's disease, we have implanted mesencephalic dopamine cells from a 7-week human embryo into the caudate and putamen of a 52-year-old man with Parkinson's disease. Fetal tissue was obtained from elective abortion. The woman and the patient with Parkinson's disease were unknown to each other. The woman gave specific consent and was not paid. The patient had a 20-year history of parkinsonism treated with multiple drug therapies including levodopa/carbidopa (Sinemet) every 2½ hours. His symptoms were worse on the left side. For 5 months prior to transplantation, the patient underwent clinical evaluations by both a neurologist and a computer system installed in his home for daily measurement of walking and hand movements. Preoperative positron emission tomographic scanning with 6-[^{18}F]fluorodopa (fluorodopa) demonstrated severe dopamine depletion bilaterally. Fetal tissue was matched to the patient for ABO blood antigens, and maternal serum was screened for hepatitis B and human immunodeficiency virus type 1 prior to surgery. Fetal tissue was implanted stereotactically throughout the caudate and putamen on the right side of the brain via 10 needle tracks. The patient was not immunosuppressed. Results 12 months after surgery showed 42% improvement in left-hand speed before the first morning dose of drug and 40% greater response to drug therapy. Right-hand speed increased 16% before drug therapy and 23% after drug therapy. Reaction time was unaffected. Walking speed increased 33% after drug administration, although walking speed before the first morning dose of drugs declined 40%. Walking speed on an all-day basis improved 17%. "On" time increased from 69% to 88% of the day. For technical reasons, preoperative and postoperative fluorodopa positron emission tomographic scans were performed at different facilities, so that results could not be directly compared. A magnetic resonance scan 5 months after surgery showed that signs of the needle tracks were still visible but that there was no enhanced signal after gadolinium injection, indicating that the blood-brain barrier was intact. These data indicate that transplants of human fetal dopamine cells may have therapeutic benefit in patients with Parkinson's disease.

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Parkinson's disease results from the death of a small number of dopamine-producing cells in the substantia nigra pars compacta. Normally, the midbrain contains about one half million dopamine cells[1] and supplies dopamine to the caudate and putamen through a rich axonal network. Parkinsonian signs appear after there is about 80% depletion of dopamine.[2] Experiments in rat and monkey models of Parkinson's disease have shown that fetal cells implanted in adult brain can grow neural processes and synthesize dopamine.[3-11] Cells of an early gestational age appear to be best. For humans, this is tissue from a first-trimester fetus.[10,11]

The promising animal experiments have led to human studies. A few patients have received implants of human fetal cells. Some information on surgical technique and clinical outcome has been presented,[12-15] including a report describing early results in our patient.[16] Although there is one detailed report describing results in two patients,[15] there is no consensus on the optimum fetal age for implant, on the brain region that should be implanted, on surgical technique, on the need for using immunosuppressants, or on the clinical usefulness of the implants. Twelve months after transplantation, our patient showed significant improvement in his disease as measured by clinical neurological examination, by a novel, home-based computer-testing system, by reductions in drug doses, and by the patient's personal assessment. Nonetheless, he continued to be bothered by freezing spells when walking and by day-to-day fluctuations of his Parkinson's disease.

In the past 2 years, there have been several hundred autologous transplants of adrenal medullary tissue performed on patients with Parkinson's disease with modest therapeutic benefits reported by some centers.[16-19] In autopsies performed on a few of these patients, survival of adrenal medullary cells has been poor.[20,21] Experiments with rat, monkey, and human tissue have found that adrenal medullary tissue requires nerve growth factor for long-term survival.[22-25] There may be an explanation for the apparent improvement found in some patients, even without cell survival. Some animal experiments indicate that adrenal medulla transplants can enhance dopamine neurite outgrowth

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even with the death of medullary cells.[26,27] Nonetheless, animal studies show that transplanted fetal dopamine cells produce behavioral effects that are superior in quality and persistence to adrenal medullary transplants.[3,4,21,28,29]

REPORT OF A CASE

The patient was a 52-year-old man with a 20-year history of Parkinson's disease treated with multiple drugs taken every 2.5 hours while awake. He was otherwise in good health. The disease first presented with left-sided findings. Over the course of time, the right side became involved but the left side remained the more severe. The patient was right-handed. Walking was made difficult by problems in initiating movement as well as by dystonic posturing of both feet. He used a cane for walking at home and used crutches when out in public. Freezing episodes while walking were his most disabling problem. Five years prior to surgery, the patient had to stop working. Because of his difficulty ambulating, the patient was classified as stage IV (Hoehn and Yahr).

Associated signs included hypophonia, progressive micrographia, loss of the ability to whistle (a favorite hobby), and chronic constipation. He had approximately one bowel movement per week. He had mild rigidity and little tremor.

He was followed up at frequent intervals by the same neurologist for 17 years. Careful titration of drugs had led to drug dosing every 2½ hours with levodopa/carbidopa (Sinemet) and multiple daily doses of trihexyphenidyl (Artane), bromocriptine (Parlodel), and amantadine (Symmetrel).

MATERIALS AND METHODS

The patient was examined by a neurologist using the Unified Parkinson's Disease Rating Scale, version 3.0.[30] Each examination tested the patient before and 1 hour after drug administration. The patient also reported on the Activities of Daily Living Scale.

In addition to the neurologic evaluations, we developed computerized timed tests of walking and hand motion that were performed in the patient's home on a daily basis beginning 5 months prior to surgery and continuing for 12 months after surgery. The microcomputer measured walking speed and left- and right-hand motion. The strategy was to test motor performance before and 1 hour after the first dose of drugs in the morning every day. In addition, 1 day per week every waking hour the patient performed these tests on an hourly basis for 15 hours.

Rapid alternating movements of each hand were measured by tapping adjacent keys of the computer keyboard with the left or right index fingers. A second test of hand motion measured reaction time and movement speed in a conditional performance task in which the patient was instructed by screen commands to move the index finger from a home key to one of two target keys on a console wired to the computer. The computer randomly selected the target key. Reaction time was the time taken to release the home key. Both hands were tested in each task. Walking speed was measured by four photocells placed along a 6-meter runway and wired to the computer. Data were analyzed in 2-month blocks by analysis of variance with Neuman-Keuls post hoc correction.

A preoperative 6-L[^{18}F]fluorodopa (fluorodopa) positron emission tomographic (PET) scan at Memorial Sloan-Kettering Cancer Center, New York, NY, using a PC 4600 positron camera demonstrated severe reduction of fluorodopa uptake bilaterally. The striatal/occipital cortex target-to-background ratio was 1.46 on both the left and right sides vs 2.69 ± 0.07 in four controls. The striatal uptake rate constant K_i was 0.0022 mL/min per gram of brain on the left and 0.0020 mL/min per gram of brain on the right, while controls averaged 0.015 ± 0.002 mL/min per gram of brain.[31] Fluorodopa was prepared by a modification of the synthesis of Luxen et al[32] to a specific activity of 120 mCi/mmole. Because of technical problems at Memorial Sloan-Kettering Cancer Center, the patient underwent a repeated fluorodopa PET scan at UCLA 9 months after transplantation.

Tissue for the transplant was obtained under criteria compatible with the guidelines developed by the Advisory Committee on Human Fetal Transplantation Research, National Institutes of Health, Bethesda, Md. The transplant protocol and the consent forms for the patient with Parkinson's disease and the donor of the fetal tissue were approved by the University of Colorado Health Sciences Center (Denver) Institutional Review Board for the Protection of Human Subjects. Human fetal brain tissue of about 7-weeks' gestation was recovered following routine suction abortion. Informed consent from the mother was obtained only after the abortion was completed. No modification in the abortion procedure was made except for the use of a sterile collection apparatus. The patient and the tissue donor were unknown to each other and the woman was not paid.

In earlier experiments, we established landmarks for dissecting mesencephalic dopamine cells from human fetal brain stem by tyrosine hydroxylase immunocytochemical staining of whole mounts of brain-stem tissue.[25] A tissue fragment (2 × 4 × 1 mm) was dissected from the rostral half of the ventral mesencephalon. The mesencephalic fragment was disrupted by trituration with a fire-polished glass Pasteur pipette in 400 μL of cold isotonic phosphate/bicarbonate-buffered saline with glucose (pH, 7.2). Aliquots of 40 μL for each transplant track were placed in individual sterile tubes. Cell viability was 65% to 87% as assessed by acridine orange and ethidium bromide staining. The total time between tissue collection and placement in the brain was 12 hours. During this time, the cells were kept at about 8°C.

We characterized the ABO blood group antigens of fetal tissue prior to transplant. In early experiments, we found that fetal red blood cells at this gestational age lacked ABO antigens but that the antigens were present on other tissues. Using an immunoperoxidase reaction on a cross section of fetal body parts, a presumptive characterization of a fetus as type A was made by specific staining of endothelial cells and other cellular elements of the fetal body with anti-A, but not anti-B, monoclonal antibodies. Because the patient was type O, the fetal tissue chosen for the transplant was unreactive to anti-A or anti-B monoclonal antibodies.

In preliminary experiments, we studied the risk of bacterial contamination of fetal material. We found no positive cultures of 17 fetuses after 2 days in aerobic and anaerobic cultures. Risks of viral infection were also addressed. Serologic investigations for human immunodeficiency virus type 1 and hepatitis B were performed on a maternal serum sample prior to fetal tissue transplantation. In the preoperative assessment, the patient was determined to be positive for cytomegalovirus. The maternal serum sample was not screened for cytomegalovirus. At delivery, routinely obtained culture samples may be positive for herpes simplex type 2 in 0.2% of women. By excluding patients with a history of genital herpes infection and by inspecting for herpes lesions, we estimated the risk of transmitting herpes to the patient to be 1:500 to 1:1000.[33]

A major question in human fetal implant therapy is whether immunosuppression is necessary. In rat and monkey studies, implants of fetal brain tissue within species are usually not rejected, even without using immunosuppressants. Using these drugs, xenografts of mouse or human can survive in rat or monkey.[10,11,34,35] However, because immunosuppressants carry some risk of morbidity and mortality primarily from infection, their use in symptomatic treatment of parkinsonism is a difficult medical decision. Only a controlled trial of fetal mesencephalic implants with and without immunosuppressants will answer this question. Because of the apparent success of fetal neural implants in monkeys without the use of immunosuppressants[11] and in the interest of patient safety, we decided not to use these drugs.

Since signs of parkinsonism were worse on the left side, we chose to implant tissue in the right side of the brain. We used the surgical strategy and methods that we had developed in a monkey model and had shown to produce behaviorally effective fetal grafts.[3] In an effort to reinnervate the whole of the caudate and putamen, 10 stereotactic needle passes were made 4 mm apart in the caudate and putamen. Because the dopamine deficiency of Parkinson's disease is most severe in the putamen, it is reasonable that the putamen should be a main focus of transplantation.[36] The rationale for track spacing at 4-mm intervals came from animal experiments of human fetal tissue placed into rats.[10,11] In these studies, dopamine cell neurites grew several millimeters, substantially reinnervating rat striatum. It was our hope that human fetal cells would have a similar growth potential in human brain and would fully restore dopamine input on one side of the brain.

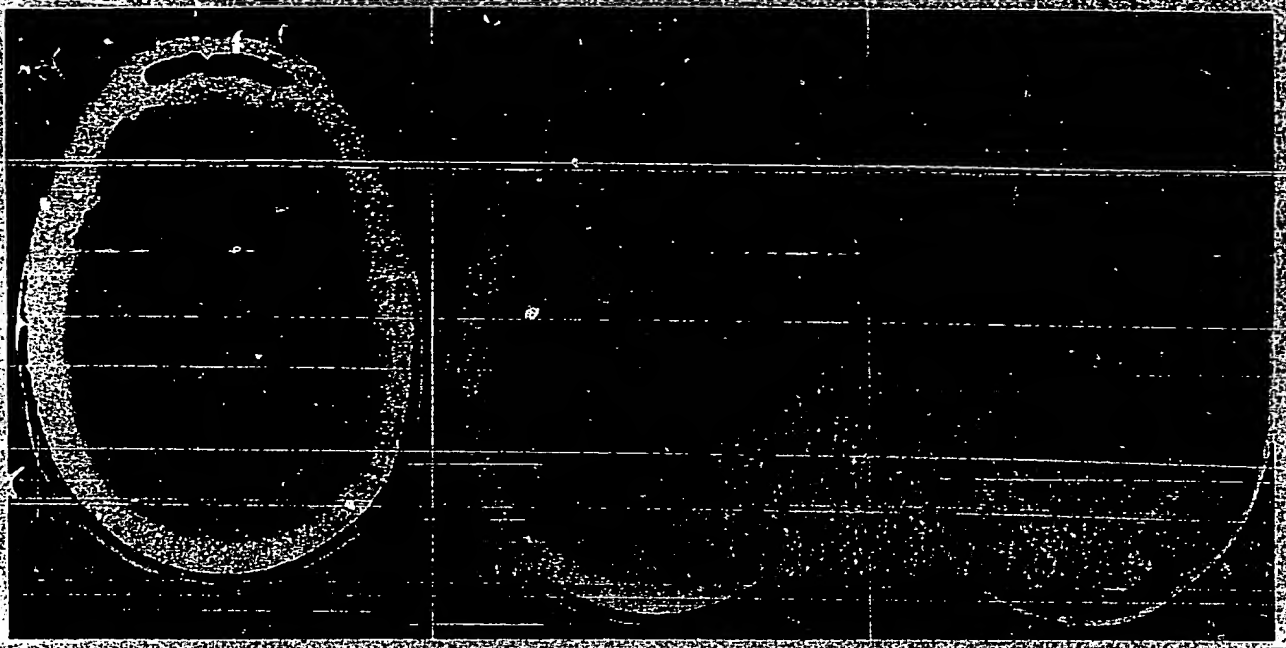

Fig 1.—Computed tomographic (CT) and magnetic resonance (MR) scans before and after fetal tissue implantation. The left-hand panel shows the CT baseline established in the putamen for transplants. A total of six needle passes, 4 mm apart, terminated at this line. The center panel shows the CT baseline through the caudate to which four needle passes were made. The right-hand panel shows a T_1-weighted MR image obtained 5 months after surgery. The arrows point to the line of the needle tracks in the caudate and putamen. Evidence of the needle tracks is still seen. Gadolinium injection showed no enhancement at 5 months, indicating that the blood-brain barrier was intact.

Table 1.—Clinical Assessment of Transplant Effects at 12 Months

	Unified Parkinson's Disease Motor Scale*		
	Pretransplant	Posttransplant	% Change†
Overall			
Before drug therapy	33	19	+42; $P < .01$
After drug therapy	17	17	0; NS
Right side			
Before drug therapy	20	15	+25; $P < .01$
After drug therapy	12	13	−8; NS
Left side			
Before drug therapy	26	12	+54; $P < .01$
After drug therapy	14	12	+14; $P < .05$

*Scale is as follows: very severe, wheelchair bound, 56; minimal disease, 8.

†Regression lines fit to neurologic examination results in postoperative period. $N = 17$; P values refer to slopes different from zero. NS indicates not significant.

Table 2.—Activities of Daily Living

Some subjective improvement 12 months after transplantation
Speech
Cutting food
Dressing
Falling
Tremor
Freezing
Walking
No subjective improvement
Salivation
Swallowing
Hygiene
Turning in bed
Sensory symptoms
Handwriting
Overall, an improvement in score while "on" of 21 to 7 (67%)

Table 3.—Medication Changes*

Medication	Pretransplant	Posttransplant	% Change
Levodopa/carbidopa (Sinemet)	900	900	0
Bromocriptine	17.5	12.5	−29
Trihexyphenidyl	8	6	−25
Amantadine	300	300	0

*Total daily dose in milligrams.

Surgery was performed using a BRW computed tomographic (CT) stereotaxic guide (Radionics, Burlington, Mass). Only local anesthesia supplemented with intravenously administered midazolam was used. With the head ring in place, the patient underwent CT scanning. Baseline coordinates were established for the caudate and putamen as shown in Fig 1. Specific trajectories were obtained for four needle tracks to be placed in the caudate and six tracks in the putamen. Trajectories were calculated to avoid the posterior limb of the internal capsule. A 3-cm trephination was placed to the right of midline at the level of the coronal suture. The entry point for the putamen and caudate tracks were at two different sites on the surface of the brain. The tracks to the putamen were vertical with reference to a coronal plane while the approach to the caudate was at an angle of 30°.

The injection cannula consisted of a 17-gauge (1.5-mm) stainless steel outer cannula with a 19-gauge inner stylet. This was inserted into the brain to the CT baseline in the caudate or putamen. The stylet was then removed and replaced with a 19-gauge infusion cannula that had been preloaded with 30 μL of tissue suspension. The tissue was slowly infused at a rate of 3 μL/min as the cannula was withdrawn. Tissue was deposited along a total distance of 15 mm in each needle track. At frequent intervals during the operation, the patient was asked to speak and move his extremities.

A postoperative CT scan showed no subdural hemorrhage or edema. The patient received phenytoin prophylactically and antibiotics parenterally on the day of surgery and for 2 days thereafter. The patient had an uneventful postoperative course and

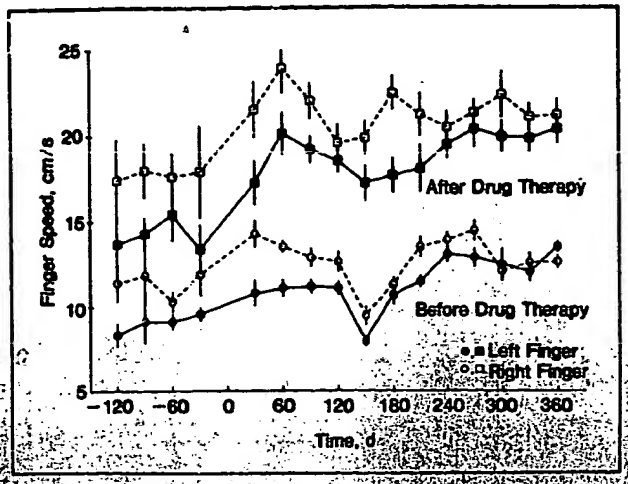

Fig 2.—Finger speed in conditional-movement task before and after the first morning dose of drugs before and after fetal tissue implantation. Speed of the left index finger (centimeters per second) is shown in the closed symbols and solid lines, while the speed of the right index finger is shown in the open symbols and dashed lines. Circles represent finger speed before drug administration and the squares the movement speed 1 hour after drug administration. Data shown are 1-month averages of daily testing as described in the "Results" section. Following surgery, finger speed increased for both the right and left fingers. Finger speed at 12 months represents a 42% increase for the left finger before drug therapy and a 40% increase after drug therapy ($P < .01$ for both values). For the right finger, the increase in finger speed was 15% before drug therapy ($P \leq .05$) and 23% after drug therapy ($P < .05$). Finger speed on the left and right sides were converging 12 months after transplantation.

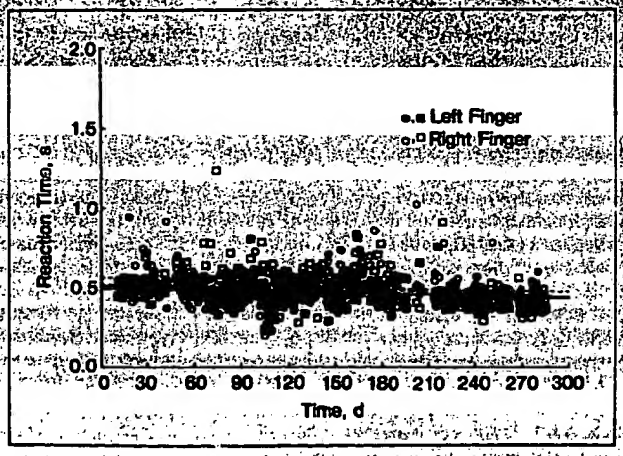

Fig 3.—Reaction time during conditional finger-movement task. The time was the delay between the appearance of the screen command to move and the lifting of the finger from a push-button switch (symbols carry the same meaning as in Fig 2). Results show that conditional reaction time was unaffected by drug therapy, the limb used, or time. As such, it represents a control for diligence.

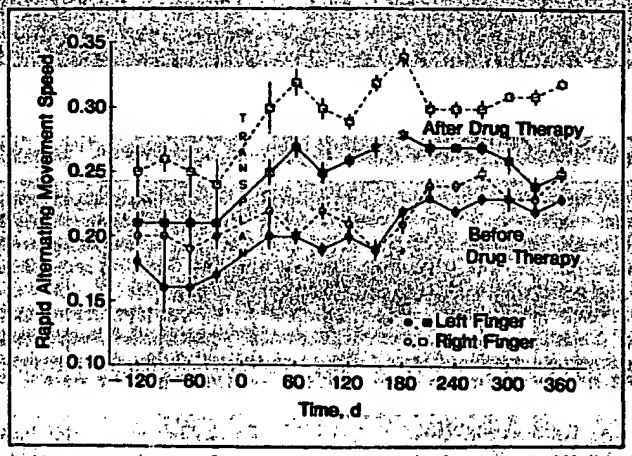

Fig 4.—Speed of rapid alternating movements (symbols carry the same meaning as in Fig 2). Data on the *y* axis are the reciprocal of the time taken to perform 10 key presses of adjacent keys on the computer keyboard. Results at 12 months show improvement for the left finger before and after drug therapy (39% and 17%, $P < .01$ for both values) as well as for the right finger (22% and 25%, $P < .01$ for both values).

was discharged on day 8 in his preoperative condition.

Because bacterial cultures of a portion of fetal tissue obtained at the time of abortion became positive for *Lactobacillus* 4 days after being placed in culture, the patient was readmitted 5 days after surgery for a lumbar puncture. The spinal fluid showed no cellular or bacteriologic evidence of infection. The patient at no time had a fever but was treated with 2 days of intravenous antibiotics and 5 additional days of oral antibiotics.

RESULTS

The patient was examined at weekly intervals postoperatively before and 1 hour after the first morning dose of drugs. He also resumed his daily testing schedule on the home computer within 2 weeks of surgery. At 1 month and 6 months after receiving the transplant, the patient underwent magnetic resonance scans, and on both occasions it was possible to see traces of the cannula tracks in the caudate and putamen (Fig 1). Gadolinium was injected during the magnetic resonance scan at 5 months. There was no contrast enhancement indicating that the blood-brain barrier was intact at that time.

Results of the neurological evaluation of the patient using the Unified Parkinson's Disease Scale are shown in Table 1. A decrease in numerical score represents improvement. Clinical examination showed improvement after surgery in his "off" state before a drug dose. There was 42% improvement in predrug performance by 11 months after surgery. Laterality of transplant effect was demonstrated in hands and feet with 54% improvement in performance on the left side compared with 25% improvement in performance on the right side. Activities of Daily Living as reported by the patient yielded a 67% improvement during "on" periods (Table 2).

Beginning at 30 days, the neurologist attempted to reduce the patient's drug doses. Judgments were made only on clinical criteria. To avoid influencing his neurologic evaluations, neither he nor the patient were told of the results of the computer test. In each case, reductions of trihexyphenidyl, bromocriptine, levodopa, and amantadine doses were associated with some loss in motor performance, as measured by the computer. To reduce the confounding effects of changes in drug dosing with changes produced by the transplant, drug dosing was restored at 7 months after the transplantation to nearly the same levels as preoperatively. At 11 months, bromocriptine doses were once again reduced because of increased movements of the head and left arm and leg. Table 3 presents the patient's drug schedule before and 12 months after surgery.

Signs of change appeared in the val-

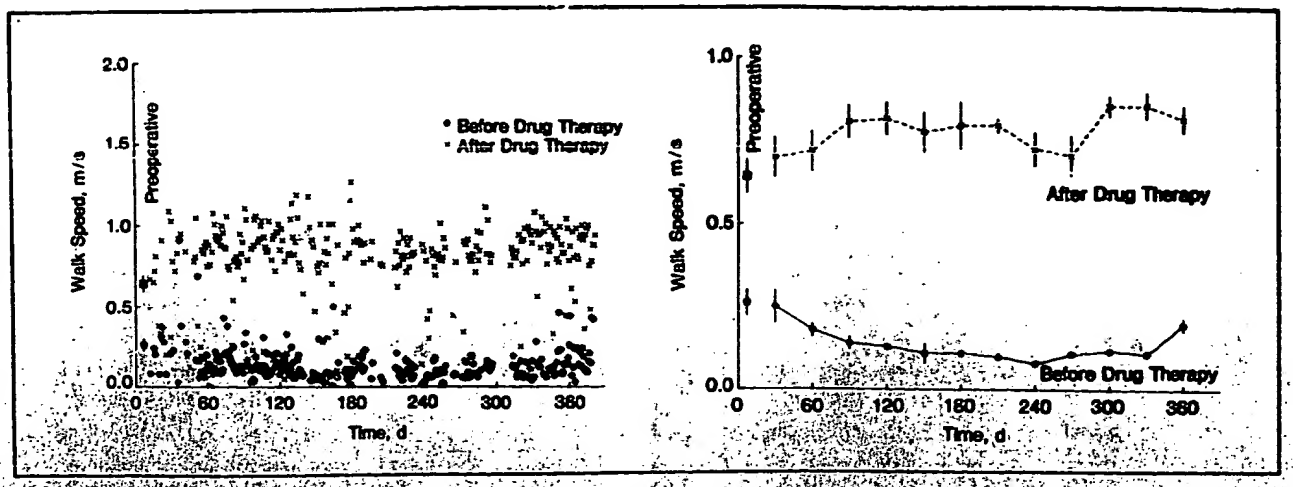

Fig 5.—Walking speed before and 1 hour after drug administration before and after surgery. The left panel shows individual daily testing results demonstrating the considerable day-to-day variability. At the left margin are the means and SEMs of the measurements made for 4 months preoperatively. The asterisks show the speed of walking before the first morning doses of drug and the Xs the walking speed 1 hour later. The panel at right shows the same data, but they are averaged at 30-day intervals. There was a significant increase in walking speed at 1 year (33%; $P < .01$). Walking speed prior to the first dose of drugs in the morning declined 40% ($P < .01$) compared with the period before surgery.

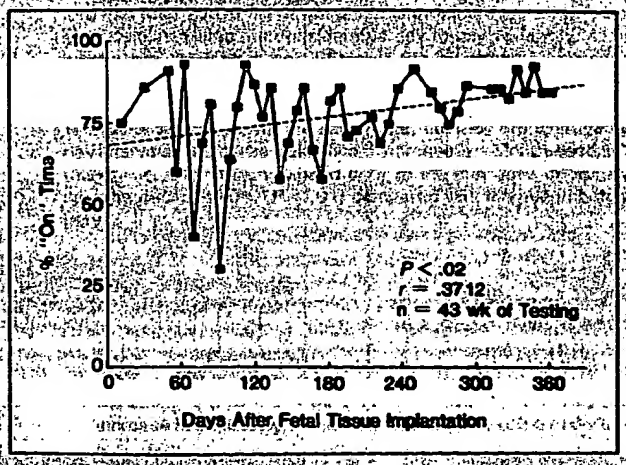

Fig 6.—"On-off" time during all-day computer testing. "On" refers to the ability to walk faster than 0.5 m/s. Overall, there has been a 17% increase in on time by 12 months after surgery (69% to 86% of the day, $P < .02$).

ues recorded on the computer test in the early weeks after the implantation and became significantly different from baseline values by 2 months after surgery. Improvement as noted by computer testing results was seen both as improvement in performance before drug therapy each morning and as an enhanced response to drug therapy. There was improvement bilaterally in hand motion through space, although left-hand improvement was greater than right-hand improvement (Fig 2). Left-finger speed increased 42% before drug administration and 40% after drug administration ($P < .01$; both values by Neuman-Keuls post hoc testing). Right-finger speed increased 15% before drug therapy ($P < .05$) and 23% after drug therapy ($P < .05$). There was no change in reaction time with either hand as shown in Fig 3.

Rapid alternating movements were tested by hitting adjacent keys of the computer keyboard with the index finger. Each hand was tested separately. As shown in Fig 4, rapid alternating movements of the left hand improved before and after drug therapy (39% and 17%, respectively; $P < .01$). The right hand showed 22% improvement before drug therapy and 25% improvement after drug therapy ($P < .01$; both values). By 12 months after surgery, his performance in both tests while using drugs was in the range expected for people without Parkinson's disease.

Walking speed 1 hour after drug use was improved 33% by 1 year after transplantation ($P < .01$) (Fig 5). By contrast, speed of walking in the early morning prior to drug therapy declined 40% ($P < .01$). We are uncertain as to why walking speed before the patient took his medications declined, especially since hand-movement speed increased even before the first morning dose of drugs.

One day per week, the patient performed the computer tests every waking hour. This testing allowed us to measure the fraction of the day the patient was "on" or "off." Walking speeds of less than 0.5 m/s were associated with the off periods. Results of the all-day testing on "on-off" times are shown in Fig 6. The patient was always off before the first dose of drugs in the morning. Thus the maximum percentage on has been 93% (14 of 15 hours during the day). The regression line in the figure shows that the patient's on time averages 86% 12 months after surgery compared with 69% at the time of surgery ($r = .37$, $n = 43$ weeks of testing, $P < .02$).

While timed tests showed a significant increase in hand-movement speed before and after the first dose of drugs in the morning, neurologic examination showed an overall improvement in motor performance in the off phase but not in the on phase. Since the clinical neurologic examination does not measure speed of movement but, rather, gauges quality of movement, computer and human testing are not equivalent.

Patient reports of Activities of Daily Living are presented in Table 2. In addition to the items on the test list, the patient described other phenomena. By 1 month after transplantation, the patient's chronic constipation resolved. He also regained the ability to whistle. There was an improvement in the clarity and force of his speech,

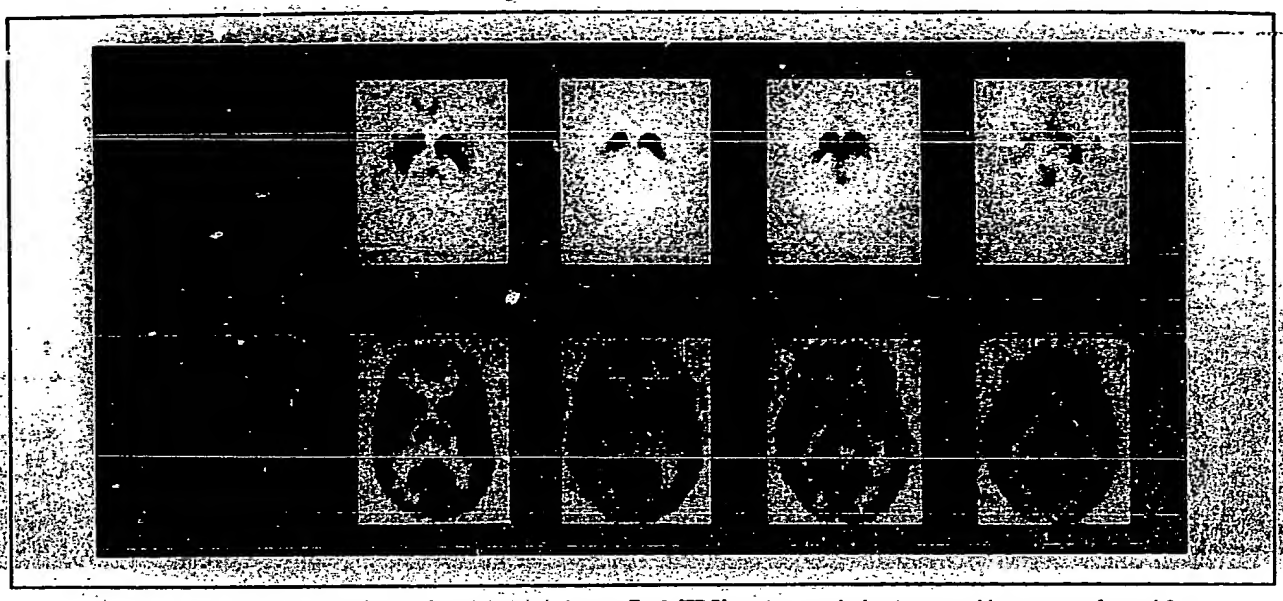

Fig 7.—6-L-[^{18}F] Fluorodopa (F-dopa) and fludeoxyglucose F-18 (FDG) positron emission tomographic scans performed 9 months after surgery. The upper series of slices are integrated uptake of fluorodopa, 60 to 120 minutes after drug injection, while the lower show FDG uptake integrated over 30 to 60 minutes. Each slice is 6.7 mm thick with the section on the left most dorsal and the right most ventral. The left side of each image is the right side of the patient's brain. Fetal implant was in the right caudate and putamen. In-plane image resolution is 8.54 mm. The FDG images provide anatomic detail against which the fluorodopa images can be assessed. In all planes, fluorodopa uptake in the caudate nucleus is substantial bilaterally. In the putamen, uptake is less and appears asymmetric, with greater uptake on the patient's right side in two of the three sections showing the putamen. Detailed calculations are presented in the "Results" section.

particularly noticeable in telephone conversations. He no longer used his cane for walking at home.

A fluorodopa PET scan was performed 9 months after surgery at UCLA using the Siemens/CTI 831 PET scanner. Uncertainties in defining the boundaries of the putamen in the most rostral slice led to data analysis only on the central slices. Results showed target-to-background ratios of 2.62 and 2.43 in the left and right caudate, respectively, while in the putamen the ratios were 2.02 and 1.74 (left and right, respectively). The K_i values for uptake at 60 to 120 minutes in the caudate were 0.0096 ± 0.0006 mL/min per gram of brain for the left and 0.0095 ± 0.0002 mL/min per gram of brain for the right, and in the putamen the values were 0.0063 ± 0.0007 mL/min per gram of brain for the left and 0.0050 ± 0.0003 mL/min per gram of brain for the right (Fig 7). Because of difference in PET scanner resolution and imaging techniques, the preoperative scan in Memorial Sloan-Kettering Cancer Center and the postoperative scan at UCLA could not be compared directly.

At 12 months, the patient was on a drug schedule that reflected a 29% reduction in the bromocriptine dose and a 25% reduction in the trihexyphenidyl dose (Table 3). Despite these reductions, the patient's hand movements and walking speed on a 15-hour per day testing basis were increased compared with preoperative testing.

COMMENT

While as many as 40 implantations of human fetal dopamine cells have been performed in England, Sweden, Cuba, Mexico, China, and now the United States, the surgical techniques used for transplantation, the age of the fetal tissue being employed, and the strategy for evaluating transplantation success are very different. In a letter to the editor, Madrazo et al[12] have described an open surgical procedure transplanting a block of fetal tissue to the caudate. Fetal tissue (13 weeks' gestation) was implanted. A second approach, by Hitchcock et al[13] has stereotactically implanted similar aged fetal tissue to the caudate. While tissue of that developmental stage may survive when transplanted as a block,[14] it is unclear that an implant into the caudate would have much therapeutic effect, since the most severe dopamine deficit of Parkinson's disease is in the putamen.[15,17]

The only detailed report of fetal implant surgery has come from Lindvall et al[15] describing results in two patients 6 months after these patients received the transplant. These investigators used a cannula (2.5 mm in diameter) to place tissue from four embryos (7 to 9 weeks' gestation) into one site in the caudate and two sites in the putamen on one side of the brain. Tissue was not immunologically characterized preoperatively, but patients were immunosuppressed with cyclosporine, prednisone, and azathioprine. Results showed that motor performance deteriorated for 1 to 2 months postoperatively but then improved beyond the preoperative level. In one patient, performance in timed tests reached a level about 20% faster than before surgery, and the patient felt there was an increase in on time. The other patient showed a lesser response and felt there was no clinical benefit to surgery. There were no changes in drug doses.

We have performed the most extensive reinnervation of the caudate and putamen reported to date. Even with implants along 10 needle tracks in the caudate and putamen, we observed no reduction in motor function perioperatively. It is possible that the larger cannula used by the Swedish investigators[15] (2.8 times the cross-sectional area of the cannula we used) accounted for the loss of performance they observed in the early postoperative period. Other differences in technique may account for the apparently greater clinical improvement observed in our patient. Tissue was held in buffered calcium- and magnesium-

free chilled Hanks' solution, while Lindvall et al[15] used unbuffered normal saline. We also attempted to fully reinnervate the caudate and putamen with needle tracks spaced 4 mm apart. The changes in motor performance we have seen in our patient have occurred over a period of months following surgery and are continuing 12 months after surgery.

Whether immunosuppression will lead to a better clinical response is uncertain. To our knowledge, we are the only group performing preoperative matching for ABO blood group antigens in an effort to reduce the risk of rejection. While the brain may be an immunologically privileged site, only a study comparing immunosuppressed and nonimmunosuppressed patients will clarify this issue.

Our quantitative measures of neurologic improvement using a novel home-based computer system have provided the richest database of any study of Parkinson's disease reported to date. Because of the objective nature of the computer measures, we believe that this method of evaluation is superior to the general neurologic examination. Since Parkinson's disease shows high variability day-by-day, only frequent measurements can provide a database by which to compare preoperative and postoperative states. By studying daily performance before and after the first dose of drugs, we were able to see if transplants influenced the basal state of Parkinson's disease or only the drug response. It may be that transplants will change drug responses more than basal motor performance. Transplantation experiments in animals support this possibility. Most animal experiments have shown changes in amphetamine- or apomorphine-induced behaviors rather than in baseline motor performance.[3,4,8-11,23,28]

Our patient has shown significant improvement in most aspects of his Parkinson's disease 12 months after implantation of fetal dopamine cells into the caudate and putamen. He has shown increases up to 42% in speed of rapid alternating movements and in hand movement through space. Movement of the left hand, the side opposite the implant, has increased in speed more than movement of the right hand. Testing while the patient was undergoing drug therapy showed hand-movement speeds to be normal. On an all-day performance basis, the patient can walk about 17% faster than before surgery and is on about 86% of the time. His walking speed after the first dose of drugs in the morning has improved 33%, although his ability to walk before his first morning dose of drugs is more impaired than it was before surgery. Reaction time was unaffected by surgery or drug therapy, as expected for the conditioned performance task we devised.[25,29] The constancy of reaction time provides a good measure of continued vigilance by the patient. His speech has improved in volume and clarity, he has regained the ability to whistle, and his chronic constipation has resolved. Drug doses have been reduced to some extent.

Magnetic resonance scanning with gadolinium injection 5 months after surgery showed no enhancement, indicating that the blood-brain barrier was intact. This result contrasts with that found after adrenal transplantation,[30] most likely because the adrenal tissue transplants contain peripheral blood vessels with permeable endothelium. With fetal neural implants, we would expect the integrity of the blood-brain barrier to be restored, as we have found.

Whether implants of fetal dopamine cells will fully reverse the signs of Parkinson's disease is uncertain. Despite the successful growth of fetal dopamine cells in animals, these implants have not been shown to reverse all aspects of the parkinsonian state. Because the process leading to Parkinson's disease is unknown and progressive, even successful fetal cell implants in man may offer only temporary benefit. Nonetheless, in a patient with severe disease we have seen substantial clinical improvement persisting for 1 year after implantation. With further refinement in cell preparation and surgical technique, it is likely that greater clinical improvement will be possible.

When this article was in press, a report from Lindvall et al[41] described improvement in a patient 5 months after a fetal neural implant. The time course and magnitude of improvement they observed was similar to that we reported at 6 mo.[16] and 1 year as noted in this article. Since submission of the 12-month data, we made the decision to optimize drug therapy. Selegiline (deprenyl) therapy (10 mg/d) was started. This therapy worsened bradykinesia and made walking more difficult. With the assumption that deterioration was caused by excess dopamine effect, we cut the dose of levodopa/carbidopa (Sinemet). Fifteen months after the implantation, levodopa/carbidopa administration has been reduced from 900 to 500 mg/d (44%), amantadine reduced from 300 to 200 mg/d (33%), trihexyphenidyl from 8 to 6 mg/d (25%), and bromocriptine from 17.5 to 10 mg/d (43%). These drug reductions have resulted in further improvement in walking speed to 1 m/s 1 hour after drug therapy, 53% faster than preoperatively. The speed of walking before the first morning dose of drugs had shown a gradual decline after transplantation. The predose walking speed has now improved to 0.3 m/s, 12% faster than preoperatively.

Drug therapy complicates the management of these patients who have received transplants. Eliminating drugs is therapeutically unacceptable for the patient with severe Parkinson's disease. Holding drug dosing constant during the posttransplant period seems like an attractive choice, but overdose as well as underdose can lead to worsening of the condition of the patient with Parkinson's disease. Since a successful dopamine cell implant may contribute to an overdose effect, transplants may worsen some aspects of the parkinsonian state. Drug therapy may slow the growth of the transplant.[42] All of these facts make studies of fetal neural implants difficult. However, as we work out the time course of growth of fetal implants (which appears to be most rapid over 2 to 6 months as we and Lindvall et al[41] have both seen), a scheduled taper of drug therapy could be initiated in anticipation of transplant effects.

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